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14. ABSTRACT

The goal of this research was to compare, in the rescue mode of treatment, the effectiveness of (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004 in reducing hearing and sensory cell loss as the result of an acute acoustic trauma that simulates blast injury to the auditory system. A shock tube was used to produce a blast injury to the cochlea in a chinchilla model. A secondary goal was to apply the same drug administration protocol to groups of animals exposed to a lower level continuous noise. Data from the treated and control animals consisted of: (1) auditory evoked potential hearing thresholds; (2) cubic distortion product otoacoustic emissions (DPOAE) input/output functions along with DPOAEs as a function of frequency (DPEgram) to estimate sensory cell function; (3) tympanograms to screen for conductive changes; (4) surface preparation histology to estimate the frequency specific sensory cell loss. Statistical analysis of the data employed a mixed model analyses of variance with repeated measures on one factor (frequency) using the SPSS Release 4.0 statistical package. Results: (1) There were no statistically significant differences between drug treated groups and controls. (2) There was a very large inter subject variability for all exposure groups.

15. SUBJECT TERMS

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I. Introduction:

The goal of this research was to compare, in the rescue mode of treatment, the effectiveness of the five drugs identified below in reducing hearing and sensory cell loss as the result of an acute acoustic trauma that simulates blast injury to the auditory system. A shock tube was used to produce a blast injury to the cochlea. A secondary goal was to apply the same drug administration protocol to groups of animals exposed to a lower level continuous noise. Each of the following substances was used to treat experimental groups of chinchillas following noise-induced trauma: (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004. Data from the treated and control animals consists of: (1) evoked potential recordings from the inferior colliculus to estimate hearing thresholds; (2) cubic distortion product otoacoustic emission (DPOAE) input/output functions along with DPOAEs as a function of frequency (DPEgram) to estimate sensory cell function; (3) tympanograms to screen for conductive changes; (4) surface preparation histology to estimate the frequency specific sensory cell loss. Statistical analysis of the data employed a mixed model analyses of variance with repeated measures on one factor (frequency) using the SPSS Release 4.0 statistical package.

II. Key Research Accomplishments:

Phase I:

- The shock tube was calibrated to produce blast waves having peak free field SPLs of 162, 165 and 169 dB using compression section charge pressures of 7, 11 or 19 psi respectively.

- The cochlear sensory cell lesion (loss) produced by 162, 165 and 169 dB peak free field SPL blast wave exposures was quantified.

- Tympanometry established that there were no significant middle ear changes resulting from 10, 169, 165 or 162 dB peak free field SPL blast exposures that would affect audiometric or histological data from experimental subjects.

Phase II:

- Control subjects were exposed for 6 hours to a 4 kHz octave band of noise at 105 dB SPL. The control groups consisted of (i) noise alone, (ii) noise plus saline injections and (iii) noise plus aqueous EDTA. Audiometric, otoacoustic emissions and histology data were obtained on these groups. Sensory cell loss and PTS were considerably less than reported in the literature for the same exposure conditions. Subsequent exposures were increased to 108 dB SPL.

- Two lesion calibration groups were run at the 108 dB noise level. In one group the sensory cell lesion approximated that found in the literature for exposures at 105 dB, in the other group the lesion and the PTS were larger.

- The following control groups of animals were exposed for 6 hours to a 4 kHz octave band of noise at 108 dB SPL: (i) noise alone, (ii) noise plus saline, (iii) noise plus aqueous EDTA, (iv) noise plus saline plus DMSO. Audiometric, otoacoustic emissions and histology data were obtained on these groups. There were no statistically significant differences among the groups. A single control group was formed from these 4 groups having an N = 28 for comparison with the drug treated groups (see below).

- The following groups of animals were exposed for 6 hours to a 4 kHz octave band of noise at 108 dB SPL and treated in the rescue mode with (i) L-NAC, (ii) D-MET, (iii) Ebselen SPI-1005, (iv) ALCAR, or (v) Src-PTK inhibitor KX1-004. Audiometric, otoacoustic emissions and histology data were obtained on these groups. There was no significant difference in hearing thresholds and sensory cell loss among the control group and the various rescue mode drug treatment groups.

Phase III:

- Animals were exposed to 10 blast waves (impulses) having peak free field SPLs of 165 dB over approximately 1.2 minutes. Peak SPL at the entrance of the external ear canal was 158 dB. Two noise only control groups were run with 20 animals/group. Variability among individual animals and between groups was large. The first control group showed significantly less trauma that did the second control group. Subsequently both groups were used as noise only controls as well as the mean of the two groups.

- The following groups of animals were exposed to 10 blast waves (impulses) having peak free field SPLs of 165 dB over approximately 1.2 minutes. Peak SPL at the entrance of the external ear canal was 158 dB. The animals were treated in the rescue mode with (i) L-NAC, (ii) D-MET, (iii) Ebselen SPI-1005, (iv) ALCAR, or (v) Src-PTK inhibitor KX1-004. Audiometric, otoacoustic emissions and histology data were obtained on these groups.

  (i) ANOVA analysis using the first control group: There was a main effect of group for PTS and for %OHC loss. However, pair wise comparisons showed that either there was no difference between the control and treated groups or that the treated groups had more PTS or %OHC loss.

  (ii) ANOVA analysis using the second control group: There was no significant difference between the control group and the treated groups.

  (iii) ANOVA analysis using the mean of the combined first and second control groups: There was a main effect of group for PTS but not for sensory cell loss. However, pair wise comparisons showed that either there was no difference between the control and the treated groups or that some of the treated groups had more PTS or %OHC loss.

- A noise only control group and three drug (L-NAC, ALCAR, D-MET) treated groups were exposed to 10 blast waves (impulses) having peak free field SPLs of 162 dB over approximately 1.2 minutes. Peak SPL at the entrance of the external ear canal was 156 dB. An ANOVA analysis showed no main effect of group for PTS, %OHC loss or %IHC loss.

III. Reportable Outcomes:

The data presented in the body of this report have not been published in the scientific literature.

IV. Conclusions:

(i) The treatment of animal subjects (chinchilla) in a rescue mode with L-N-acetylcysteine, D-Methionine, Ebselen SPI-1005, Acetyl-L-carnitine or Src-PTK inhibitor, KX1-004 following an acoustic trauma produced by a 4 kHz octave band of noise at 108 dB SPL for 6 hours showed no statistically significant reduction in permanent threshold shift or sensory cell loss.

(ii) The treatment of animal subjects (chinchilla) in a rescue mode with L-N-acetylcysteine, D-Methionine, Ebselen SPI-1005, Acetyl-L-carnitine or Src-PTK inhibitor, KX1-004 following an acute

acoustic trauma produced by exposure to either 165 dB or 162 dB peak SPL free field blast waves showed no statistically significant reduction in permanent threshold shift or sensory cell loss.

In summary the experiments produced negative results i.e., there was no advantage to the treatment of animals with any of the 5 drugs used. None of the 5 drugs was effective in reducing the extent of blast wave induced hearing trauma or continuous noise induced hearing trauma. Any effects that the drug treatment may have had was likely obscured by the large individual variability in response to the noise exposures. It should be noted that the negative results presented in this report are at odds with most of the results presented in the scientific literature.

V. Body:

Contents:

List of Terms and Abbreviations

AAT.....Acute Acoustic Trauma

ANOVA.....Analysis of Variance

A/D.....Analog/Digital

AEP.....Auditory Evoked Potential

ALCAR.....Acetyl-L-Carnitine

dB .....deciBell

daPa.....dekaPascals

DMSO .....Dimethyl Sulfoxide

DPOAE.....Distortion Product Otoacoustic Emissions

DPgram.....Distortion Product as a function of frequency

D-MET.....D-Methionine

EDTA ..... Ethylene diamine tetraacetic acid

EEG..... ElectroEncephaloGraph

ECV.....Equivalent Canal Volume

EtOH.....Ethyl Alcohol

f.....frequency

I.M.....IntraMuscular

I.P.....IntraPeritoneal

I/O.....Input/Output

IHC.....Inner Hair Cell

kHz.....kiloHertz

L-NAC.....L-N-Acetylcysteine

mOs.....milliOsmole

OBN.....Octave Band Noise

OsO_4.....Osmium tetroxide

OHC .....Outer Hair Cell

PTS .....Permanent Threshold Shift

psi.....pounds per square inch

SPL .....Sound Pressure Level

A. Background:

The increased metabolic activity associated with high-level noise exposure is known to result in the excessive production of reactive oxygen species (ROS) and other free radicals in various cell populations of the cochlea including the sensory cells. In October of 2005 a symposium entitled "Pharmacologic Strategies for Prevention and Treatment of Hearing Loss and Tinnitus" was held in Ontario, Canada. Comprehensive and up to date reviews on the effects that a variety of drugs have on noise-induced hearing loss (NIHL) can be found in the proceedings of this symposium [Canlon et al., (eds.) 2007]. Following this symposium the U.S. Department of Defense held a workshop that focused on evaluating the pharmacologic approaches to reducing NIHL as they might apply to the military condition. It was clear from the discussions at this meeting that hearing loss as a result of acute blast exposure was a serious problem in current military operations, resulting in casualty status for large numbers of personnel. One of the results of this meeting was the identification of five drugs that were at a Technology Readiness Level (TRL) of 4 or greater (Dept. of Defense 2005). These drugs were: N-acetyl-L-cysteine (L-NAC), Ebselen, D-Methionine (D-MET), Acetyl-L-Carnitine (ALCAR) and a Src Inhibitor, KX1-004. Each of these drugs was shown to reduce NIHL and cochlear sensory cell loss following some noise exposures in various animal model systems. The workshop attendees suggested that (a) an impartial critical review of all the available data on these five drugs relating to hearing be performed and (b) that a "head to head" comparison, of the efficacy of these drugs be undertaken using the same protocol and species. The review of the published literature through 2006 was performed under contract from Science Applications International Corporation (Hamernik, 2006). The research reported below represents a response to Item (b) above. It should be recognized that since the publication of the 2005 symposium proceedings there have been several publications that haven shown the efficacy of combined drug therapy in reducing acoustic trauma from continuous noise as well as from blast wave exposures (e.g., Ewert et al. 2012; Campbell et al. (2011); Coleman et al. 2010; and others.

B. Methods:

(1) Shock (blast) wave generation and calibration: A 14.8-foot long, compressed air driven shock tube was used to produce the blast wave stimulus. The shock tube has a 6 X 6 inch cross-section with 6-foot exponential horn having a 4 X 4 foot opening at the exit. A schematic (not to scale) of the shock tube and exposure setup is shown in Figure 1. Clear acetate film (Grafix Plastics) with a thickness of 0.003 in. was used to separate the compression and expansion chambers. A pneumatically actuated needle was used to puncture the acetate film thereby initiating shock wave formation and propagation. Shock wave strength and thus the peak sound pressure level (SPL) of the impulse noise stimulus is directly proportional to the pressure in the compression section.

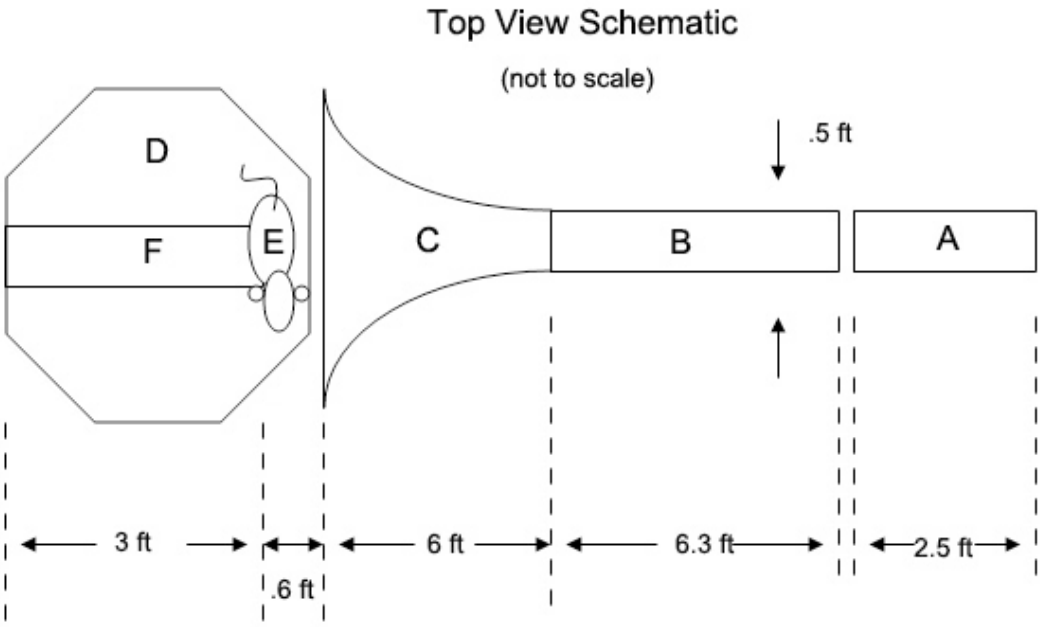


Figure 1. A schematic view of the shock tube and the exposure setup. (A) Compression section. (B) Expansion section. (C) Exponential horn. (D) Reverberant chamber.
(E) Subject. (F). Subject mounting platform.

Experimental animals were exposed in a reverberant chamber constructed from ¼ inch aluminum plate and shaped in the form of a dodecahedron approximately 3 feet in 'diameter' positioned at the exit of the shock tube horn. Each animal was restrained in a leather, full body harness with the pinna fixed to insure an unobstructed external meatus. All animals were subjected to an otoscopic examination. The shock wave was calibrated by measuring the peak SPL within the reverberant chamber at the location that the subject would occupy during the exposure (free field measurement). Measurements were made both with and without the animal present using a 1/8-inch Bruel & Kjaer (B&K) microphone (type 4138-B-006). The microphone was connected to a B&K measuring amplifier (type 2610) via B&K cable (AO 0428-D-050). A B&K pistonphone (type 4220) was used to calibrate the microphone. The microphone was mounted at the location of the animal's experimental ear for calibration purposes only. The shock and calibration waveforms were digitized at a sampling rate of 48 kHz and saved in computer files using a National Instruments DAQ Card 6024E.

(2) The 4 kHz octave band noise generation and calibration: The 4 kHz noise was created using an Electro-Voice Xi-1152/94 speaker and amplifiers (Model P1200 and P2000). The sound field was calibrated and recorded using a B&K, ½ inch microphone (Model 4134), amplified by a B&K 2610 measuring amplifier and digitized by an analog-to-digital/digital-to-analog converter (Model PCI-6221, National Instrument Inc.). The sound level of the noise was monitored using a Larson Davis 814 sound level meter equipped with a ½ inch microphone. The design and digital generation of the acoustic signal is detailed in Hsueh and Hamernik (1990, 1991) and Hamernik et al. (2003). The signal was sampled at 48 kHz in 16 bits with a recording duration of 5.5 minutes. Several segments of the signal were recorded in the center of each cage and saved on hard disc for off line analysis. The SPL and spectral data were obtained from these recordings using programs developed using MATLAB. The SPLs across cages varied within approximately +/- 1 dB. Animals to be exposed were confined, but free to move, in a bank of 4 stainless steel cages having overall dimensions 22 X 12 X 6 inches mounted 34 inches below the speaker. The size of the individual animal's holding cage was 11 X 6 X 6 inches. Freedom of movement was limited and the animals typically remained immobile for the duration of the exposure. The noise was a 4 kHz OBN presented at either 105 or 108 dB SPL for 6 hours.

(3) Subjects: The chinchilla was used as the experimental subject. Each animal was anesthetized [IM injection of Ketamine (12.86 mg/kg) Acepromazine (0.43 mg/kg) and Xylazine (2.57 mg/kg) or Telazol (20 mg/kg)] and made monaural by the surgical destruction of the left cochlea. A bipolar platinum electrode (Plastics Products Co., Model MS303/S) with a 7.5 mm probe length and a 2.5 mm ground was implanted, under stereotaxic control, into the region of the left inferior colliculus for single-ended recordings of the auditory-evoked potentials (Henderson et al., 1973; Salvi et al., 1982). During electrode implantation, on-going EEG activity to auditory click stimuli was monitored in order to ensure the adequate and consistent placement of the electrode. A postoperative analgesic agent (buprenorphine HCL; 0.01 mg/kg every 12 hours) was administrated as needed. The animals were allowed to recover for 2 weeks before evoked potential testing began. Subjects were excluded from the study for any one of the following conditions: vestibular abnormalities following surgery; preexposure thresholds that were above laboratory norms. [Note: Laboratory norms for the auditory evoked potential (AEP) thresholds are based on over 1500 normal animals and are in agreement with the accepted thresholds for the chinchilla (Fay, 1988).] When the temporal bones (bullae) were removed the middle ear was checked for the presence of fluid or adhesions and if present, data from that animal was rejected. All animals were screened prior to inclusion in an experiment. If an animal's threshold exceeded one standard deviation from the norm at more than one frequency in the direction of poorer thresholds the animal was rejected.

(4) Tympanometry: Admittance tympanograms were obtained on a number of blast exposed animals using a Grason-Stadler Inc. otoadmittance instrument (GSI Model 39 version 1). An otoscopic examination was performed prior to tympanometry to evaluate the outer ear canal and tympanic membrane. The admittance tympanograms were recorded in an audiometric suite (Industrial Acoustics) using a 226-Hz probe tone (85.5 dB $\pm$ 2.0 dB) while the animal was awake and restrained in a leather harness to minimize movement. Admittance magnitude was measured while air pressure in the external ear canal was decreased from +200 daPa to - 400 daPa. The average admittance value obtained from three tests before and immediately following noise-exposure was determined for three parameters: (a) equivalent canal volume (ECV - cm^3); (b) peak-static middle-ear admittance (Peak - cm^3) and (c) gradient (the width of the tympanogram at 50% of the admittance, i.e., compliance - daPa.). The purpose of this testing was: (i) to screen animals for any pre-existing middle ear abnormality and (ii) to rule out tympanic membrane perforations or ossicular discontinuity as a confounding factor in post-exposure results.

(5) Hearing threshold testing procedure: Hearing thresholds were estimated on each animal using the AEP. The animals were awake during testing and restrained in a yoke-like apparatus to maintain the animal's head in a fixed position within the calibrated sound field (Blakeslee et al., 1978). AEPs were collected to 20-ms tone bursts (5-ms rise/fall time) presented at a rate of 10 per second. A general-purpose computer was used to acquire the evoked response data and control the frequency, intensity, and timing of the stimulus. The electrical signal from the implanted electrode was amplified (50,000X), filtered (30 to 3,000 Hz) and led to the input of an A/D converter where it was sampled at 20,000 samples per second (50-μs period) over 500 points to obtain a 25-ms sampling window. Each sampled waveform was analyzed for large amplitude artifacts and if present, the sample was rejected from the average and another sample taken. Averaged AEPs were obtained from 250 presentations of the 20-ms signal. Each waveform was stored for later analysis.

Thresholds were measured using an intensity series with 5 dB steps at octave intervals from 0.5 to 16 kHz. Threshold was determined to be one-half step size (2.5 dB) below the lowest intensity that showed a response consistent with the responses seen at higher intensities. The average of at least three separate threshold determinations at each frequency on different days was used to define the pre-exposure audiogram. At least 30 days after the noise exposure, final audiograms were obtained using the same protocol as followed for the pre-exposure audiogram. Permanent threshold shift (PTS) was defined as the difference between the post-exposure and pre-exposure thresholds at each test frequency.

(6) Distortion product otoacoustic emissions (DPOAE): Cubic distortion products ($2f_1$-f_2) were measured in the ear canal of the awake but restrained animal with the Mimosa Acoustics Inc. HearID (R3.4) Module otoacoustic emissions test instrument using CUBeDIS (v2.40) software. The HearID instrument was connected to the Etymotic ER-10C sound probe via a probe adapter cable. The DPOAE was measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively where $f_2/f_1 = 1.22$ and $L_1 = L_2 + 10$ dB. Pre and post exposure data collection consisted of two parts: (a) DPOAEs as a function of the f_2 primary frequency (DPgrams) at $L_1 = 55$ and 65 dB and (b) DPOAE input/output (I/O) functions at six DPOAE frequencies (i.e., the DPOAE at a given frequency as a function of the L_2 primary tone level).

The DPgram data were collected at 25 points (1/6 octave steps) across the approximately 0.8 to 12.5 kHz range of f_2 and plotted as a function of f_2. The averaging time was constant at 2 s. For each animal 3 sets of DPgrams were collected on three different days preexposure and 30 days post exposure. The mean of the 3 individual DPgrams along with the mean noise floor was plotted as a function of f_2.

DPOAE input/output (I/O) functions were collected at DPOAE frequencies ($2f_1$-f_2) of approximately 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 kHz in 5 dB steps of L_2. Actual DPOAE frequencies measured are in parentheses in bold type: (**985** Hz, f_1=1266; f_2 =1547), (**2015** Hz, f_1=2578; f_2=3141), (**3000** Hz, f_1=3844; f_2 = 4688), (**3984** Hz, f_1=5109; f_2=6234), (**6001** Hz, f_1=7688; f_2=9375) (**8016** Hz, f_1=10266; f_2=12516). For each animal 3 sets of I/O functions and the noise floor were collected pre and 30 days post exposure and the mean of each set of three was accepted as that subject's emission levels. The DPOAEs were collected at approximately the same times during the experimental sequence as the AEP audiograms. The average of the three pre- and three 30-day post-treatment sets of DPOAE measurements were used to establish permanent treatment effects.

(7) Drug treatment protocol: The 5 drugs used in the rescue treatment of noise-induced hearing loss were: (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004. All 4 kHz noise exposures began at 8 a.m. and terminated at 2 p.m. while the blast wave exposures took place between 8 and 9 a.m. Immediately at the end of each exposure the animals were given an I.P. drug injection. Eight (8) hours later a second injection was given. Over the next 4 days, injections were given at 8 a.m. and 4 p.m. Thirty (30) days following the exposure PTS and DPOAEs were collected and the animals were then euthanized for histology. The doses administered were:

(i) L-N-acetylcysteine (L-NAC): L-NAC was dosed at 325 mg/kg from a 20% commercial preparation (Mucomyst, Hospira Inc.). This preparation contains 200 mg/ml L-NAC and 0.5 mg/ml EDTA, an antioxidant in a saline solution.

(ii) Acetyl-L-Carnitine (ALCAR): ALCAR (Sigma-Aldrich, Cat. #A-1509-1G) was dosed at 100 mg/kg from a 25mg/ml solution in sterile normal saline.

(iii) D-Methionine (D-MET): D-MET (Sigma-Aldrich, Cat. #M-9375) was dosed at 200 mg/kg from a 30 mg/ml solution in sterile normal saline.

(iv) Ebselen SPI-1005: Ebselen (Sigma-Aldrich, Cat. #E-3520) was dosed at 16 mg/kg from a 50 mg/ml solution in DMSO.

(v) Src-PTK inhibitor, KX1-004: The Src inhibitor (Kinex Pharmaceuticals) was dosed at 50 mg/kg from a 100 mg/ml solution in DMSO.

(8) Surface preparation histology: Following the last test protocol, each animal was euthanized under anesthesia at either 30 min or 30 days after noise exposure. [The exceptions are two groups of animals in the Phase I experiments that were euthanized either immediately or 10 days after exposure.] The right auditory bulla was removed and opened to gain access to the cochlea for perfusion. The stapes was removed and the round window membrane ruptured. A fixation solution consisting of 2.5% glutaraldehyde in veronal acetate buffer (final pH=7.3, 605 mOs) was perfused through the cochlea. After 24-h of fixation the cochlea was postfixed in 1% OsO_4 in veronal acetate buffer, washed in buffer and dehydrated to 70% ETOH. The entire basilar membrane was dissected free and mounted in glycerin on glass slides for a surface preparation, light microscopic assay of the entire organ of Corti sensory cell population (Engstrom et al., 1966). Inner and outer hair cell (IHC, OHC) populations were determined over consecutive 0.24-mm sections of the organ of Corti, beginning at the apex as a function of distance along the cochlear duct. Baseline sensory cell populations were established within octave lengths of the cochlea using a large population (N = 30) of normal chinchillas. These figures agree with the normative data published by Bohne et al. (1982). Sensory cell counts, which eventually yield cytocochleograms, were performed at magnifications of 500X using a Zeiss-Nomarski interference contrast universal microscope. A cell was counted as missing when the cell body was not present. In animals that have survived more than 30 days after trauma, the location of missing cells is usually well marked by characteristic phalangeal scar figures at the level of the reticular lamina or a squamous cell scar on the basilar membrane. Cell counts were averaged over 0.24-mm lengths of the organ of Corti as measured along a reference line established by the junction of the inner and outer pillar cells at the highest level of the reticular lamina. This line also established the length of the organ of Corti. A frequency-place map established by Eldredge et al. (1981) was used to superimpose frequency coordinates on the length coordinate of the cochleogram so that audiometric data could be directly related to the sensory cell populations along the length of the cochlea. All the light microscopic analysis and graphics were accomplished directly from the microscope through a computer system with appropriate morphometric software. For purposes of this report, sensory cell population data is presented as group averages (in percent

missing) computed over octave-band lengths of the cochlea centered at 0.125 through 16 kHz in 8 octave steps. Total mean IHC and OHC losses over the entire cochlea were also obtained for each experimental group.

(9) Statistical analysis: Evoked potential permanent threshold shifts and inner/outer hair cell losses in octave-band lengths of the cochlea were compared among the groups of control and drug treated animals using a two-way analysis of variance (ANOVA). The probability of a type I error was set at 0.05. Statistically significant main effects of frequency were expected and found in all of the following analyses because of the frequency-specific nature of the audibility curve of the chinchilla and the noise exposure stimulus. For this reason main effects of frequency are not addressed in the following presentation of results. Pair wise post-hoc analyses (Tukey/Kramer test) were performed to establish any significant effects between the control and individual drug treatment groups.

C. Results:

(1) Phase I: Shock tube generated blast wave source calibration and quantification of the cochlear lesion.

(a) Statement of work taken from the approved proposal: *The shock tube exposure and subsequent cochlear lesion will be 'calibrated'. This will entail the exposure of at least 50 animals. Only histology and tympanometry will be performed on these 50 animals. Approximately half will be killed immediately following noise exposure in order to define the initial sensory cell lesion and the remainder killed after 30 days to estimate the extent of the final stable lesion. The objective is to establish an exposure protocol that will produce, with low variability, a lesion that is typical of a blast-induced trauma. Peak SPLs for this calibration process will vary between 155 and 165 dB peak SPL. The 'best' level will be selected. More animals will be used as needed to arrive at the parameters necessary to create a consistent lesion. Histology on animals killed immediately following exposure will establish whether or not the lesion is typical of blast trauma.*

(b) Calibration of the shock tube: The peak SPL at the position of the animal as a function of the shock tube driver pressure (psi) is shown in Figure 2. Peak SPL in the free field varied from approximately 155 dB to 168 dB over the 5 to 21 psi range of shock tube compression section charge pressures that were used. The square symbols represent the three peak SPLs that were chosen to calibrate the shock wave induced lesion in the chinchilla blast trauma model. Figure 3 shows examples of the waveforms that were recorded for the three exposure conditions used for the lesion calibration both with and without the animal in the exposure position. The spectrum of the impulse produced by the 11psi driver pressure and measured both

with and without the animal in place is shown in Figure 4. The energy of the impulse peaks around 100 Hz. A more detailed exposition of the shock wave calibration data for the 7, 11 and 19 psi charge pressures is shown in Figures 5 through 10. These figures illustrate the repeatability of the shock wave noise stimulus both in the free field and with the animal in the exposure configuration. Also, noticeable in these figures (see e.g., Fig. 3) is the increased reverberant character of the impulse when measured with the animal in place compared to that of the free field. It should also be noted that the animal is oriented with the right (experimental) ear on the down stream side of the advancing shock wave, i.e., in the sound shadow of the head.

(c) Calibration of the blast-induced lesion: Cochlear histology (a quantification of the number of missing inner and outer sensory hair cells) was completed on 94 subjects. Each subject received 10 shock wave exposures in approximately 1.2 minutes. The animals were oriented with the long axis of the body parallel to the advancing shock wave and with the right (experimental) ear in the down stream position i.e., the left ear was exposed to the free field wave while the right ear was in the sound shadow of the head. During the brief exposure the animal was completely restrained in a leather harness and the pinna was secured so that the entrance to the external canal was unobstructed. Five groups of animals were exposed. The distribution of animals was as follows:

(i) Thirty (30) animals were exposed to the168 dB peak SPL free field waves (19-20 psi charge pressure) and euthanized 30 days post exposure.

(ii) Twelve (12) additional animals received the same exposure as in (i) and were euthanized immediately after exposure.

(iii) Twenty (20) animals were exposed to the 165 dB peak SPL free field waves (11 psi charge pressure) and euthanized 30 days post exposure.

(iv) Thirteen (13) additional animals received the same exposure as in (iii) and tympanometry was performed pre and immediately following the exposure to verify the status of the middle ear. Animals were euthanized 30 days post exposure and histology performed.

(v) Nineteen (19) animals were exposed to the 162 dB peak SPL free field waves (7 psi charge pressure) and euthanized 30 days post exposure.

The group mean cochleograms for groups (i), (iii) and (v) identified above are shown in Figure 11. These figures show the percent IHC and OHC loss as a function of the frequency specific location on the basilar membrane. Each datum point represents the group mean percent cell loss computed over an octave band length of the basilar membrane. There is, as expected, a systematic reduction in sensory cell loss as the peak SPL is decreased. In all three cochleograms the peak of the OHC loss is in the 1.0 to 2.0 kHz octave band region of the cochlea. The total number of IHC and OHC lost along with the standard error of the mean

(in parenthesis) is also given in each panel. Cochleograms for individual animals in the three groups are shown in Figures 12 through 14. These individual cochleograms provide some appreciation of the variability typically seen in blast wave exposures in particular and noise exposures in general (see also the section below on the tympanometric data).

The individual cochleograms from the 12 animals exposed to the 161 dB peak SPL wave (19-20 psi charge pressure) and euthanized immediately following the exposure (i.e., @ T = 0) are shown in Figure 15. The interesting feature in 9 of these animals is the immediate (i.e., within ~5 min of exposure to the first impulse) appearance of missing sensory cells in the region of the cochlea that develops the maximum loss by 30 days post exposure (see Fig.11). In several of the animals there are relatively substantial losses while in several others there was relatively little loss. The acute lesion is likely the result of mechanically induced damage to the organ of Corti (Hamernik et al., 1984). Such lesions have been shown to grow in extent as neighboring cells develop necrotic or apoptotic changes and are lost over a period as long as 10 days post exposure. Across animals, different stress/strain responses in the tight cell junctional complexes along the reticular lamina may be a significant factor in the excessive variability seen in blast wave exposures. This, coupled with the uncontrolled genetic background of the chinchillas likely contributes to the variability that is evident in Figures 12-15.

(d) Pre and post noise exposure tympanometry: As part of the Phase I work pre and post exposure tympanometry or a post exposure visual evaluation of the middle ear was performed on a total of 76 animals. The objective was to: (i) establish a normative data base for middle ear variables using tympanometry; (ii) determine if the planned exposures would cause middle ear problems that could contribute to variability. From past work (Hamernik and Henderson, 1974) the experimental ear facing upstream and oriented with the plane of the external ear canal parallel to the plane of the advancing shock wave was the position most likely to cause middle ear problems. For this reason the right, downstream ear was chosen as the experimental ear. A number (22) of these animals were euthanized immediately after the post exposure tympanometry was completed; the remainder was euthanized 30 days post-exposure and histology performed. The reason for euthanizing the animals immediately post-exposure was to visually ascertain the status of the tympanic membrane and the middle ear and to determine the initial status of the cochlea (see Fig. 15).

(i) Admittance tympanograms using a 226-Hz probe tone were obtained in 63 normal unexposed animals in order to establish normative values and to assess test-retest reliability. The average admittance value obtained from three tests in each animal was determined for three admittance variables: equivalent canal volume (ECV cm^3), peak-static middle-ear admittance (Peak - cm^3), and gradient* (compliance - deca-Pascals)]. The results are shown in Table 1. (* Gradient = the width of the tympanogram at 50% of the admittance.)

Table 1**.** The normative group mean (N = 63) descriptive statistics for the admittance variables : equivalent canal volume (ECV cm^3), Peak (cm^3), and Gradient (daPa).

	ECV(cm^3)	Peak (cm^3)	Gradient (daPa)
Mean	0.92	1.43	83.65
Standard Deviation	0.17	0.85	25.23
Standard Error	0.03	0.08	2.56

Test-Retest reliability: The consistency of admittance results showed a strong correlation between each test administration. Correlation coefficients (Pearson's r) for test-retest reliability were as follows: ECV (r =0.93); Peak (r = 0.87); and Gradient (r = 0.95). The test-retest difference (i.e., first and second test vs. second and third test) for each admittance value was not statistically significant ($p < 0.05$).

(ii) Admittance tympanograms with a 226-Hz probe tone were obtained pre and immediately post exposure in 44 chinchillas exposed to 10 impulses at 168 dB peak free field SPL (19-20 psi shock tube compression section charge pressure). The animals were oriented with the experimental right ear in the down stream position, i.e., in the sound shadow of the head. The average admittance value obtained from three tests both before and immediately following the exposure in each animal was determined for the three admittance variables: ECV, Peak, and Gradient. The results are shown in Table 2. There was no significant difference in admittance resulting from noise exposure for the ECV, Peak, or Gradient variables (t-test, $p > 0.05$)

Table 2. The group mean pre and post exposure descriptive statistics for the admittance variables from 44 chinchillas exposed to 10 impulses at 168 dB peak free field SPL (19-20 psi shock tube compression section charge pressure).

	ECV(cm^3)	Peak(cm^3)	Gradient (daPa)
	Pre/Post	Pre/Post	Pre/Post
Mean	0.9/0.92	1.38/1.47	82.3/92.7
Standard Deviation	0.15/0.23	0.42/0.2	17.5/12.2

(iii) Admittance tympanograms with a 226-Hz probe tone were obtained pre and immediately post exposure in 9 chinchillas exposed to 20, 165 dB peak SPL free field impulses (11 psi charge pressure). The animals were oriented with the experimental ear in the up stream position. (Note: at the 11 psi charge pressure the upstream ear receives a 165 dB peak SPL impulse while at the down stream ear the impulse peak is 158 dB.) The average admittance value obtained from three tests both before and immediately following

the exposure in each animal was determined for three admittance variables: ECV, Peak, and Gradient. The results are shown in Table 3. There was no significant difference in admittance resulting from noise exposure for the ECV, Peak, or Gradient variables (t-test, $p > 0.05$)

Table 3. The group mean pre and post exposure descriptive statistics for the admittance variables from 9 chinchillas exposed to 20 free field impulses at 165 dB peak SPL (11 psi shock tube compression section charge pressure).

	ECV (cm^3)	Peak (cm^3)	Gradient (daPa)
	Pre/Post	Pre/Post	Pre/Post
Mean	0.98/1.29	1.6/1.72	76.9/90.2
Standard Deviation	0.15/0.74	0.42/0.2	17.5/12.2

(iv) Admittance tympanograms with a 226-Hz probe tone were obtained pre and post exposure in a second group of 10 chinchillas exposed to 10, 165 dB peak SPL free field impulses (11 psi charge pressure). The animals were oriented with the experimental ear in the up stream position. The average admittance value obtained from three tests both before and immediately following the exposure in each animal was determined for three admittance variables: ECV, Peak, and Gradient. The results are shown in Table 4. There was no significant difference in admittance resulting from noise exposure for the ECV, Peak, and Gradient variables (t-test, $p > 0.05$)

Table 4. The group mean pre and post exposure descriptive statistics for the admittance variables from 10 chinchillas exposed to 10 free field impulses at 165 dB peak SPL (11 psi shock tube compression section charge pressure).

	ECV(cm^3)	Peak (cm^3)	Gradient (daPa)
	Pre/Post	Pre/Post	Pre/Post
Mean	0.96/0.94	1.51/1.7	93.3/95.7
Standard Deviation	0.12/0.09	0.48/0.59	40.1/42.0

(v) Admittance tympanograms with a 226-Hz probe tone were obtained pre and post exposure in 13 chinchillas exposed to 10, 158 dB peak SPL impulses (11 psi charge pressure). The animals were oriented with the experimental ear in the down stream position. The average admittance value obtained from three tests both before and immediately following the exposure in each animal was determined for three admittance variables: ECV, Peak and Gradient. The results are shown in Table 5. There was no significant

difference in admittance resulting from noise exposure for the ECV, Peak, and Gradient variables (t-test, $p > 0.05$)

Table 5. The group mean pre and post exposure descriptive statistics for the admittance variables from 13 chinchillas exposed to 10 impulses at 158 dB peak SPL (11 psi shock tube compression section charge pressure).

	ECV(cm^3)	Peak(cm^3)	Gradient(daPa)
	Pre/Post	Pre/Post	Pre/Post
Mean	1.2/1.3	1.37/1.45	75.0/74.2
Standard Deviation	0.25/0.31	0.43/0.29	25.8/26.7

A note on variability: The group mean cochleogram for the 13 animals discussed above (i.e., Table 5 the 158 dB peak SPL group) is shown in Figure 16 and the 13 individual cochleograms that comprise this group are shown in Figure 17. Compare these results with the lesion calibration data shown in Figure 11. The exposure condition for both sets of animals was identical. The mean and individual animal results in Figures 16 and 17 are completely atypical of what would be expected based on the data shown in Figure 11. All animals were exposed to the shock tube one at a time, in the same position with the pinna secured so that the external canal was not impeded in any way. Each animal had a preexposure otoscopic examination of the external canal. The shock tube calibration was shown to be very stable and should not have been a factor in the differences between these two groups. Tympanometric results could not offer any insight. The middle ear function appeared normal pre and post exposure. No viable explanation for this degree of variability can be offered except for the standard escape of 'tough and tender ears', i.e., genetic differences.

(e) Summary of the visual examination of the middle ear: The following 22 animals were euthanized less than 45 min post exposure. The right bulla on each was removed and opened allowing for a visual inspection of the tympanic membrane and middle ear. Middle ears on all animals appeared normal. Only the following changes in the tympanic membrane were noted:

(i) Exposure: 20 X, at 168 dB peak free field SPL (N=3); experimental ear in the down stream position. Two animals showed inflamed radial fibers in the tympanic membrane. One animal showed petechia within the membrane.

(ii) Exposure: 10 X, at 168 dB peak free field SPL (N=4); the experimental ear of 3 of the animals was in the down stream position (161 dB peak). Two of these animals showed inflamed radial fibers in the tympanic membrane. One was normal. The fourth animal with its ear in the up stream position (168 dB) showed a spindle shaped perforation of the tympanic membrane. Tympanometric data on this animal could

not be obtained due to an inability to get a seal of the probe in the external canal. This was the only animal to present with a tympanic membrane perforation.

(iii) Exposure: 3 X, at 168 dB peak free field SPL (N=3); experimental ear in the up stream position. Two animals showed inflamed radial fibers in the tympanic membrane. One showed petechia within the membrane.

(iv) Exposure: 1 X, at 168 dB peak free field SPL (N=3); experimental ear in the up stream position. All three tympanic membranes appeared normal.

(v) Exposure: 20 X, at 165 dB peak free field SPL (N=4); experimental ear in the up stream position. Two animals showed petechia in the tympanic membrane. Two were normal.

(vi) Exposure: 10 X, at 165 dB peak free field SPL (N=5); experimental ear in the up stream position. Three animals showed inflamed radial fibers in the tympanic membrane. One had petechia and one was normal.

(f) Conclusions based on the data obtained from Phase I: Based on the lesion calibration and tympanometry results; (i) the shock wave exposures for Phase III will take place at a free field peak SPL = 165 dB (i.e., at a shock tube compression section driver pressure of 11 psi) and (ii) considering that tympanometry and visual examination following exposures that were considerably more severe than those that will be used in subsequent experiments (i.e., 158 dB SPL at the down stream ear), it is unlikely that middle ear trauma is a significant contributor to variability in histological or AEP results.

(2) Phase II: Rescue drug treatment following 4 kHz octave band noise exposures.

(a) Statement of work taken from the approved proposal: *In this phase of the study the following substances will be used in a rescue mode to treat the experimental groups of chinchillas that have been exposed to a 4.0 kHz octave band of noise for 6 hours at 105 dB SPL: (1) L-N-acetylcysteine (L-NAC); (2) D-Methionine (D-MET); (3) Ebselen SPI-1005; (4) Acetyl-L-carnitine (ALCAR) and (5) Src-PTK inhibitor, KX1-004. Five reference/replication experimental groups and 3 control groups will be run with 8 animals /group. The drug treatment protocol is as described in the original proposal and is similar to that used in several published studies. These 8 groups will serve three functions: (a) partially replicate published work. (b) Establish a reference treatment outcome from a less traumatic exposure to which the AAT results can be compared. (c) Determine the most effective two of the five drugs in reducing hearing loss from an often-used test exposure.*

(b) Control groups exposed to the 105 dB SPL, 4 kHz noise: The following control groups were exposed to the 6-hour, 105 dB SPL, 4 kHz OBN and a complete data set consisting of AEP audiograms, DPOAEs and histology were acquired:

(i) Eleven (11) animals were exposed to only the 105 dB noise (noise only control group).

(ii) Six (6) animals were exposed to the same 105 dB, 4 kHz noise but in a reconfigured speaker set up.

(iii) Eight (8) animals were exposed to the same 105 dB, 4 kHz noise and were administered saline injections on the same schedule as described for the drug administration protocol (Saline control group).

(iv) Four (4) animals were exposed to the same 105 dB, 4 kHz noise and were given aqueous EDTA injections on the same schedule as described for the drug administration protocol (EDTA control group).

The spectrum and individual cage noise levels are shown in Figure 18(A) for the 4 kHz OBN at the 105 dB SPL, exposure level.

The group mean results for the noise only control group [(i) above] are shown in Figures 19 to 21. The group mean PTS [Fig. 19(A) & (B)] varied from a maximum of 27 dB at 4 kHz to between 7 and 20 dB at the other test frequencies except 0.5 kHz where there was no PTS. Sensory cell loss peaked in the 4 kHz octave band with a 40% loss of outer hair cells [Fig. 19(C)]. The corresponding decrements in the DPOAE grams and I/O functions are shown in Figs. 20 & 21. The DPOAE grams generally reflect the profile of PTS and OHC loss while the I/O functions also show a decreased output but with a somewhat different frequency profile. One objective of the Phase II experiments was to use an exposure that would produce similar PTS and sensory cell loss seen in a number of the published drug studies that used the same 4kHz noise. To achieve this objective we used the same 4 kHz OBN exposure conditions that were used by other laboratories anticipating that the exposure would cause a comparable degree of cochlear trauma. We used as our specific 'trauma' reference point the data of Kopke et al., (2002). The hearing and sensory cell loss that we recorded, as described above, was substantially less than that reported for the same exposure conditions in the Kopke et al., (2002) paper. Despite careful checking and monitoring of the acoustic environment we could not uncover a reason for this difference. To confirm that the noise did indeed cause a severe hearing loss at least at the end of the exposure, DPOAEs were run, immediately following the exposure, on four of the animals from the group of 11 shown in Figure 19. These DPOAE results are shown in Figure 22. The emissions were essentially eliminated across most of the test frequency range confirming that the noise did initially have a severe effect on virtually the entire OHC system.

As a consequence of the low PTS and cell loss, we reconfigured our speaker and cage setup [exposure (ii) identified above], recalibrated the system and exposed 6 more animals to the 4 kHz noise at 105 dB.

Noise level variations across cages in both systems were acceptably small and there was no discernable difference in the acoustics of the original and changed configurations. The group mean results are shown in Figures 23 to 25. The OHC loss is small and peaks in the 4 kHz octave band region of the cochlea. The OHC loss in this group is about half that found in the original 105 dB noise only control group (see Fig. 19). The PTS, however, in the original and reconfigured set up group were very similar (compare Figs. 19 and 23). Comparing the DPOAE data in Figures 20 and 21 with Figures 24 and 25 for these two groups confirms the 4 kHz location of maximum noise effect but the decrement in DPOAE is considerably greater in the group exposed in the reconfigured set up. The difference in DPOAEs in the two groups suggests that the cochleogram may not accurately reflect the functional status of the OHC system. Since the exposure calibration for these two groups was identical the two groups were collapsed into a single noise only control group with N = 17. The combined group data are shown in Figures 26 through 28. [Note: The mean PTS in Fig. 23 is based on N = 5. The AEP electrode came loose in one animal.]

Since we anticipated using the 105 dB exposure for the Phase II experiments we started to run a noise plus saline and a noise plus EDTA control group [Groups (iii) and (iv) described above] before the results of groups (i) and (ii) discussed above were complete. The results for the saline control group (N = 8) are shown in Figures 29 through 31 and for the EDTA control group (N = 4) in Figures 32 through 34. Within each of the three control groups the data are consistent, that is, the cell loss, PTS and DPOAE decrement co-vary. The DPOAEs from the EDTA control group show a loss over a much greater frequency extent than would be expected from the profile of OHC loss, suggesting, as discussed above in the (reconfigured) noise only control data, that the OHCs that are present are not functioning normally.

Comparing the combined noise only control groups (i) and (ii) with the saline control group (iii) and the EDTA control group (iv) yields an inconsistent data set. Ideally there should be no significant differences among the control groups. However, all three control groups were different with the EDTA control group exhibiting the largest PTS and OHC lesion while the saline control group showed the least. Individual cochleograms for all the animals exposed to the 105 dB SPL, 4 kHz OBN discussed above are shown in Figures 35, 36 and 37. The presentation of the individual cochleograms in this format provides a good visual appreciation of the amount of variability that was experienced with the 105 dB, 4 kHz OBN. A statistical analysis of these data was not performed.

Based on the results presented above a decision was made to increase the level of the 4 kHz exposure to 108 dB SPL in an effort to more closely replicate the sensory cell losses reported in the literature and to perhaps reduce the variability.

(c) Lesion calibration using the 108 dB, 4kHz noise: The spectrum and noise levels in the individual cages are shown in Fig. 18(B) for the 4 kHz OBN at the 108 dB SPL exposure level. With reference to the

problem discussed above (i.e., the limited severity/extent of the noise-induced lesion produced by the 105 dB noise exposure) two groups of animals were exposed to the 6-hour, 4 kHz OBN at 108 dB SPL. One group of thirteen (13) animals had pre and post exposure DPOAEs measured and were euthanized for histology 10 days post exposure. No audiometric data were collected on this group. A second group of 20 animals were exposed and euthanized 30 days post exposure and only histological data was collected. The objective of these two groups was to get, relatively quickly, some indication of the extent of the pathology that could be expected from the 108 dB exposure before investing considerable time in running animals through the complete experimental protocol. The group mean cochleogram and DPOAE data for the 13 animals from the first group are shown in Figures 38 through 40. The OHC loss shown in Figure 38 exceeds 60% in the 4 kHz octave band region and the loss extends from the 2.0 through the 16.0 kHz region of the basilar membrane. This noise-induced loss is comparable with previously published data (Kopke et al. 2002) using a 105 dB SPL exposure level. The DPOAE data shown in Figures 39 and 40 show a severe decrement in or elimination of emission output between 1.0 and 16 kHz, reflecting the region of the OHC loss. However, as discussed earlier the loss of emissions is larger than what would be expected on the basis of the OHC loss suggesting that cells that were counted as present were not functioning normally. Some appreciation for the histological variability seen in these animals is shown in Figure 41 where the 13 individual animal cochleograms are presented in a descending order of the number of missing OHCs.

The group mean (N = 20) cochleogram for the second group euthanized 30 days after exposure is shown in Figure 42 and individual subject cochleograms are shown in Figure 43. On average there was 70 to almost 90% OHC loss in the 2 to 16 kHz region of the cochlea. IHC losses were smaller at 15 to 30 % between 4 and 16 kHz. The OHC lesion is significantly greater in the second group than it is in the first. While the difference in postexposure survival times might have been a mitigating factor the noise-induced lesion should have stabilized by day 10. This difference in cell loss between the two groups again raises the issue of the large variability associated with noise effects research discussed in previously. Based on the lesion calibration results discussed above the decision was made to use the 108 dB noise level for the exposures in the Phase II experimental drug treatment experiments.

(d) Control groups exposed to the 6-hour, 108 dB SPL, 4 kHz octave band noise: The entire experimental protocol (i.e., PTS, DPOAEs, & histology) was completed on the following four control groups. They were: (i) Noise only control, N = 8; (ii) Noise plus saline control group, N = 8, injected post exposure twice daily for 5 days with normal saline; (iii) EDTA control group, N = 6, injected post exposure twice daily for 5 days with an aqueous solution of EDTA*; (iv) Saline plus DMSO control group, N = 6, injected post exposure twice daily for 5 days with saline plus DMSO*.

[*Note: Some published data using L-NAC treatment, used a commercial (Mucomyst) aqueous

preparation containing EDTA. The Src and Ebselen were put into solution using DMSO. Both EDTA and DMSO have anti-oxidant/anti-inflammatory properties. As a result the SOW was modified to include control groups for both EDTA and DMSO. This required an additional 4 animals.]

(i) The noise only control group (N=8): Group mean data are shown in Figures 44 to 46. Mean PTS varied from about 48 to 55 dB between the 2 and 16 kHz test frequencies. The corresponding OHC loss varied from 70 to 80 % while IHC loss peaked at about 35% at 8 kHz (Fig. 44). Distortion product otoacoustic emissions were effectively absent from 2 kHz and above and depressed between 1 and 2 kHz (Figs. 45 and 46). The sensory cell loss is similar to the lesion calibration group (N = 20) shown in Figure 42.

(ii) The noise plus saline control group (N = 7/8): The group mean data are shown in Figure 47. (The group mean PTS data are based on 7 animals. The evoked potential electrode on one animal came loose. All other mean data for this group are based on 8 animals.) Mean PTS varied from approximately 40 to 48 dB across the 2 to 16 kHz test frequency range. The corresponding OHC loss varied from 60 to 80% with about a 28 % IHC peak loss at 8 kHz (Fig. 47). DPOAEs were essentially absent across the entire test frequency range (Figs. 48 and 49).

(iii) The noise plus aqueous EDTA control group (N = 6). Group mean data are shown in Figures 50 to 52. PTS varied from approximately 48 to 55 dB across the 2 to 16 kHz test frequency range. The corresponding OHC loss varied from 65 to 85% with about a 29 % IHC peak loss at 8 kHz (Fig. 50). DPOAEs were effectively absent from 2 kHz and above (Figs. 51 and 52).

(iv) The noise plus saline plus DMSO control group (N = 6). Group mean data are shown in Figures 53 to 55. PTS varied from approximately 33 to 48 dB across the 2 to 16 kHz test frequency range. The corresponding OHC loss varied from 60 to 72% with about a 37 % IHC peak loss at 8 kHz. (Fig. 53). DPOAEs were effectively absent or severely depressed from 2 kHz and above (Figs. 54 & 55).

Individual cochleograms for all the animals in the 4 control groups are shown in Figures 56 to 59. These figures provide an indication of the extent of the variability encountered in the 4 control groups. A graphical comparison of the PTS and cell loss data for these 4 control groups is shown in Figure 60. The ANOVA analysis of the data shown in Figure 60 indicated that there were no statistically significant differences (no main effect of group) among the 4 different control groups [PTS ($F = 0.737$, $p = 0.5406$); IHC ($F = 0.549$, $p = 0.6536$); OHC ($F = 0.176$, $p = 0.912$)]. Therefore the groups were collapsed into a single noise control group with N = 28 for use in comparisons with the drug treated groups. The result of combining these groups is shown in Figures 61 to 63. PTS varies from approximately 40 to 50 dB between 2 and 16 kHz while OHC losses amounted to between 60 and 75 percent. DPOAEs were eliminated between 2 and 16 kHz and depressed between 1 and 2 kHz. Overall, for the N = 28 group mean data, the audiometric, histological and emission data were congruent.

(e) Drug treatment of chinchillas in the rescue mode following an exposure to a 4.0 kHz octave band of noise for 6 hours at 108 dB SPL. The 5 drugs used were: L-N-acetylcysteine (L-NAC); D-Methionine (D-MET); Ebselen SPI-1005; Acetyl-L-carnitine (ALCAR) and Src-PTK inhibitor, KX1-004. All noise exposures began at 8 a.m. and terminated at 2 p.m. at which time the animals were given an I.P. drug injection. Eight (8) hours later a second injection was given. Over the next 4 days, injections were given at 8 a.m. and 4 p.m. Thirty (30) days following the exposure AEPs and DPOAEs were collected and the animals were then euthanized for histology.

(i) L-N-acetylcysteine (L-NAC): L-NAC was dosed at 325 mg/kg from a 20% commercial preparation (Mucomyst, Hospira Inc.). This aqueous preparation contains 200 mg/ml L-NAC and 0.5 mg/ml EDTA, an antioxidant. The group (N=8) mean results of this treatment are shown in Figures 64 to 67. PTS in the 2 to 16 kHz regions varied from approximately 35 to 50 dB while OHC loss varied from about 48 to 70 %. At the lower primary intensities the DPOAEs were effectively eliminated across the entire test frequency range. In the 1 to 2 kHz range at the higher intensity (L_1 = 65 dB) the DPOAEs were severely depressed. Figure 67 shows the cochleogram for each individual animal in the group. The figure gives some appreciation of the variability within the group.

(ii) Acetyl-L-Carnitine (ALCAR): ALCAR (Sigma-Aldrich, Cat. #A-1509-1G) was dosed at 100 mg/kg from a 25mg/ml solution (sterile normal saline). The group (N=8) mean results of this treatment are shown in Figures 68 to 71. The mean PTS varied from approximately 40 to 55 dB across the 2 to 16 kHz range of test frequencies. OHC loss varied from 55% at 2 kHz to a maximum of 95% at 4 kHz. DPOAEs were abolished or severely reduced across the entire range of test frequencies. The individual animal cochleograms are shown in Figure 71.

(iii) D-Methionine (D-MET): D-MET (Sigma-Aldrich, Cat. #M-9375) was dosed at 200 mg/kg from a 30 mg/ml solution (sterile normal saline). The group (N=8) mean results of this treatment are shown in Figures 72 to 75. The mean PTS varied from approximately 50 to 58 dB across the 2 to 16 kHz range of test frequencies with 20 to 30 dB PTS at 0.5 and 1.0 kHz. OHC loss at 2 kHz and above was 100% with up to 60% loss at 1.0 kHz and the lower frequencies. DPOAEs (Fig. 73 and 74) were abolished across the entire range of test frequencies. The individual animal cochleograms are shown in Figure 75.

(iv) Ebselen SPI-1005: Ebselen (Sigma-Aldrich, Cat. #E-3520) was dosed at 16 mg/kg from a 50mg/ml solution (DMSO). The group (N=8) mean results of this treatment are shown in Figures 76 to 79. The mean PTS varied from approximately 37 to 42 dB across the 2 to 16 kHz range of test frequencies. OHC loss varied from approximately 40 to 65% across the 2 to 16 kHz region of the cochlea. DPOAEs were severely reduced between 2 and 10 kHz especially at the lower intensities. Below 2 kHz the decrement in the DPOAEs was less reflecting the OHC profile of loss. The individual animal cochleograms are shown in Figure 79.

(v) Src-PTK inhibitor, KX1-004: The Src inhibitor (Kinex Pharmaceuticals) was dosed at 50 mg/kg from a 100 mg/ml solution (DMSO). The group (N=9) mean results of this treatment are shown in Figures 80 to 83. The mean PTS varied from approximately 35 to 45 dB across the 2 to 16 kHz ranges of test frequencies. OHC loss varied from approximately 55 to 70% across the 2 to 16 kHz region of the cochlea. DPOAEs were severely reduced between 2 and 10 kHz especially at the lower intensities. Below 2 kHz the decrement in the DPOAEs was less reflecting the OHC profile of loss. The individual animal cochleograms are shown in Figure 83.

In summary, for all 5 drug treated groups the mean effect of the noise was quite similar, i.e., large PTS at and above 2 kHz with much less PTS at 0.5 and 1.0 kHz. DPOAEs were typically reduced across all frequencies with a profile of loss that generally reflected the loss of outer sensory cells although the complete loss of DPOAEs with less than total loss of OHCs should be recognized. The sensory cell loss generally began to increase at 2 kHz and either reached a maximum at 4 kHz and decreased at the higher frequencies or remained high at frequencies above 2 kHz.

A graphical comparison of the frequency specific magnitudes of the group mean PTS, OHC loss and IHC loss for the control and drug treated groups is shown in Figures 84 to 86. The ANOVA indicated that there was no statistically significant ($p < 0.05$) main effect of group for PTS ($F = 1.54$, $p = 0.190$); OHC loss ($F = 2.29$, $p = 0.057$); or IHC loss ($F = 2.09$, $p = 0.785$). That is, there was no significant difference in the hearing and sensory cell loss among the control group and the various rescue mode drug treatment groups exposed to the 4 kHz, OBN noise at 108 dB SPL, although the OHC loss did approach significance. Similarly, there was no interaction of group by frequency [PTS ($F = 1.42$, $p = 0.091$); IHC ($F = 1.40$, $p = 0.07$); OHC ($F = 1.43$, $p = 0.057$)]. Post hoc, pair wise comparisons were made using the Tukey/Kramer test. There were significant differences in PTS between D-MET and the control, Ebselen, L-NAC and Src Inh groups with D-MET showing the largest PTS. There were no differences between paired groups for IHC loss but there were significant differences in OHC loss between D-MET and all the other five groups with D-MET showing the greatest OHC loss.

The final analysis of the Phase II data set looked at the total number of missing IHC and OHC over the entire length of the basilar membrane for each group. A graphical comparison of the group mean total number of missing sensory cells for the control group and the 5 drug treated groups is shown in Figure 87. The ANOVA for the OHC loss indicated no statistically significant difference among the groups ($F = 2.28$, $p = 0.0571$). There was also no statistically significant difference in the IHC loss ($F = 2.0327$, $p = 0.0871$). In the above frequency specific as well as total analyses of the OHC loss, the ANOVA approached significance at the 0.05 level likely as a result of the relatively larger OHC loss seen in the group treated with D-MET.

(3) Phase III: Rescue drug treatment following 158 dB peak SPL blast wave exposures.

(a) Statement of work (revised*): *Each of the 5 drugs (L-NAC; D-MET; Ebselen SPI-1005; ALCAR and Src-PTK inhibitor, KX1-004) will be used to treat 5 groups of animals exposed to the 158 dB peak SPL impulses (blast waves), i.e., the peak SPL produced by discharging the shock tube at an 11psi charge pressure. Each group will consist of 20 subjects for a total of 100 drug treated animals. An additional 20 animals will serve as a noise only control group. These 6 groups will follow the same testing and treatment protocols as used in the Phase II, 4 kHz octave band exposures, i.e., treatment will begin immediately post exposure (T=0). The single 'best performing' drug will then be administered to another group exposed to the same blast waves but treatment will begin 1- hour post exposure (T=1). The best treatment schedule (i.e., T=0 or T=1 hour post) will then be repeated with each of 2 different drug doses in two additional exposed groups of 20 animals. This will require 3 additional groups or 60 subjects. Thus the revised SOW will utilize data from 180 subjects that have completed the entire protocol.*
[*A revision of the original Phase III SOW was required since there were no two drugs in Phase II of this work that performed 'best' in a rescue mode of treatment.]

(b) Control groups exposed to the 158 dB peak SPL blast waves:

The exposure: All animals were exposed to 10 blast waves (impulses) having a peak free field SPL of 165 dB over a period of approximately 1.2 minutes. The impulse was produced by the discharge of a shock tube with a compression section charge pressure of 11 psi. The animals were exposed with the right ear in the down stream position where the peak SPL measured at the animal's ear was 158 dB. Each animal was restrained in a whole body leather harness and the pinna of the right ear was secured with tape to insure an unobstructed external canal. Each animal was positioned at the same location within the hard walled enclosure.

The data that comprise the noise only controls were derived from the following:

(i) Two noise only control groups (N = 20/group) were run and a complete data set consisting of AEP audiograms, DPOAEs and histology were acquired. The second noise only control group was run, as will be explained below, in order to obtain a clearer picture of the extent of the untreated between group variability. (Note: the SOW for Phase III specified only one, N = 20, noise only control group.)

(ii) The Phase I lesion calibration exposures: For these animals only cochleogram data were obtained.

(iii) An additional 10 animals were exposed and only cochleogram data were obtained.

Figure 88 shows the group mean PTS and cochleogram for the first group of twenty (20) noise only control animals exposed to the 158 dB peak SPL impulses (blast waves). PTS varied from approximately10 to 25 dB across the range of AEP test frequencies. A broad profile of outer hair cell (OHC) loss between 1

and 8 kHz was recorded with a peak loss in the 2 kHz octave band of ~ 45 %. Inner hair cell (IHC) losses were considerably smaller. Across the entire cochlea there were approximately 1500 OHCs missing. The DPOAE data are shown in Figures 89 and 90. There is up to a 20 dB depression in the DPOAE output across a broad range of frequencies and primary intensities that generally reflects the degree and extent of the PTS. It should be noted that, in general, there were no inconsistencies among the audiometric, emission and histological data for the individual animals.

The PTS and cell loss results of this control group are compared to the group of animals (see Fig. 11 & 13) used in the Phase I shock tube lesion calibration in Figure 91. The lesion calibration animals were exposed in the same configuration and to the same blast waves as the noise only control animals. There is a considerable difference in the severity of the lesion. Cochleograms for the individual animals comprising the Phase III noise only control group are shown in Fig. 92. There is substantial variability in the response of the animals to the blast exposure despite stringent control of the exposure conditions. However, it should be noted that the range of the individual subject OHC loss in the control and calibration cochleograms is quite similar (compare Fig. 13 & 92). Nine (9) subjects shown in Fig. 92 showed little or no effect of the exposure as compared to only one in the calibration exposures (Fig. 13). The output of the shock tube has been checked several times and has proven to be very consistent. Each animal is handled in the same way and each is checked to be certain that the external canal is clear at the time of exposure. The pinna is secured so that it cannot occlude the external canal. Middle ear effects can be eliminated as a contributing factor since the pre exposure emissions and AEP thresholds are normal and the Phase I tympanogram results showed no changes in middle ear function at the levels used for the controls as well as at higher levels. Given the differences between the lesion calibration data and the first noise only control group a second noise only control group (N = 20) was run. These two groups are referred to below as control groups #1 and #2.

The group mean AEP, DPOAE, cell loss and individual cochleogram results from the second noise only control group are shown in Figures 93 through 96. The PTS varied from ~ 25 to 45+ dB across the 1.0 to 16.0 kHz test frequencies. DPOAEs were essentially eliminated between 1 and 4 kHz and severely depressed at the other frequencies. There was up to 80 % OHC loss at 1.0 and 2.0 kHz with smaller (20 to 60 %) losses at the other frequencies. There was a general agreement among the AEP, DPOAE and %OHC loss sets of group mean data as well the individual animal data. In the first control group where there was considerable variability with 9 animals showing little or no sensory cell loss. In the second control group only 4 animals showed little or no sensory cell loss (Fig. 96).

In a final attempt to look at the issue of variability an additional group of 10 animals were exposed to the same 165 dB SPL free field blast waves and only cochleograms obtained. (Note: this group was also not part of the SOW.) A comparison of the group mean OHC loss for the two blast wave noise only control groups (#1 and #2) and the Phase I blast wave lesion calibration along with the additional 10 lesion

calibration animals is shown in Fig. 97. The group mean %OHC loss seen in these additional 10 subjects (open circles in Fig. 97) fell between the losses found in the two noise only control groups. Clearly there is considerable variability in the OHC sensory cell lesion that was produced by the blast wave exposure. The standard error of the mean for a collection of noise only control groups may be too large to distinguish the effects of a drug therapy unless much larger sample sizes are used.

The group mean PTS and sensory cell loss data for the two noise only control groups are shown in Fig. 98 along with the mean (filled triangle) of the two data sets. There are more than 15 dB differences in the group mean PTS between the two control groups and as much as 40% differences in %OHC loss at the most affected frequencies (1 & 2 kHz). The ANOVA indicated that there was a significant difference in PTS ($F = 4.805$, $p = .0346$) and OHC loss ($F = 4.936$, $p = .0323$) between the two control groups but no significant difference in IHC loss ($F = 2.044$, $p = .1610$). This situation poses a problem of how to establish an appropriate control group to which the drug treated animals can be compared. Several options exist:

(i) Use the original noise only control group #1 (solid square symbol in Fig. 98) that exhibited the least PTS and sensory cell loss.

(ii) Use the second noise only control group #2 (open square symbol in Fig. 98) that exhibited the most PTS and sensory cell loss and in which the %OHC loss was very similar to the original calibration data (open square symbol in Fig 97).

(iii) Use the mean PTS and % cell loss of the two noise only control groups shown in Fig. 98. This would yield relatively large sample sizes (i.e., N = 40) for the PTS, %IHC and %OHC loss noise only control group.

(c) Drug treatment of chinchillas in the rescue mode following an exposure to 10, 165 dB peak SPL free field impulses.

(i) Rescue treatment of blast exposed animals with L-NAC: Figures 99 through 102 present the group (N = 20) mean results of the animals treated with L-NAC (325 mg/kg, i.p.) twice daily for 5 days following an exposure to 10, 165 dB peak SPL, free field, blast waves (11 psi shock tube charge pressure). The PTS varied between approximately 30 and 40 dB across the 1 to 8 kHz test frequencies with a corresponding 70 to 80% loss of outer hair cells (Fig. 99). Emissions were severely reduced but not eliminated at the higher frequencies and intensities (Figs. 100 and 101). The group mean permanent changes in thresholds, emissions and cell loss are much greater than in the first noise only control group (Figs. 88 through 90) and similar to the second noise only control group (Figs. 93 through 95). The rescue treatment with L-NAC did not appear to reduce trauma from the blast exposure. Cochleograms from the individual animals in the L-NAC treated group are shown in Figure 102.

(ii) Rescue treatment of blast exposed animals with D-MET: Figures 103 through 106 present the group (N = 20) mean results of the animals treated with D-MET (200 mg/kg, i.p.) twice daily for 5 days

following an exposure to 10, 165 dB peak SPL, free field, blast waves (11 psi shock tube charge pressure). The PTS (Fig. 103) varied from approximately 18 dB at 0.5 and 16 kHz to 30 dB at 8 kHz. The frequency specific OHC losses were somewhat greater than that seen in the first noise only control group (Figs. 88 through 90) with up to 45% loss across the 0.5 to 8 kHz frequency range. The frequency specific profile of OHC loss was, however, similar to that seen in the first noise only control subjects discussed above (Fig. 88). The decrement in DPOAEs (Figs. 104 and 105) generally reflected the PTS and sensory cell loss and was also greater than that measured in the first control group (Figs. 89 and 90). Individual cochleograms for the subjects in this group are shown in Fig. 106.

(iii) Rescue treatment of blast wave exposed animals with ALCAR: Figures 107 through 110 present the group (N = 20) mean results of the animals treated with ALCAR (100 mg/kg, i.p.) twice daily for 5 days following an exposure to 10, 165 dB peak SPL, free field, blast waves (11 psi shock tube charge pressure). The PTS (Fig. 107) varied between approximately 20 and 40 dB across the entire test frequency range with a maximum loss at 2 kHz. There was a corresponding 65 to 70% loss of outer hair cells (Fig. 107) in the 1 and 2 kHz octave band regions. Emissions (Figs. 108 and 109) were severely reduced or eliminated across the entire range of test frequencies. The individual cochleograms for the ALCAR treated animals are shown in Figure 110. The range of OHC loss in the individual cochleograms is comparable to that of the two noise only control groups (Figs. 92 and 96) but the group mean profile of OHC loss is similar to that of the second noise only control group (Fig. 93).

(iv) Rescue treatment of blast wave exposed animals with the Src Inhibitor: Figures 111 through 114 present the group (N = 21) mean results of the animals treated with the Src Inhibitor (50 mg/kg, i.p.) twice daily for 5 days following an exposure to 10, 165 dB peak SPL, free field, blast waves (11 psi shock tube charge pressure). There was a maximum PTS of approximately 40 dB at 2 kHz and 20 to 35 dB PTS at the other test frequencies (Fig. 111). Outer hair cell loss also peaked at 2 kHz with 75% missing cells in the 2 kHz octave band. The profile of OHC loss was very similar to that found in the original lesion calibration group and in the 2nd noise only control group (see Fig. 93 and 97). Distortion product emissions (Figs. 112 and 113) were essentially eliminated from 0.8 through 4.0 kHz and severely reduced above 4.0 kHz. The cochleogram for each animal in the group is shown in Fig. 114. The cochleograms are arranged in a decreasing order of OHC loss severity. As with all previous blast exposed groups, individual subject sensory cell loss varies from minimal through quite severe.

(v) Rescue treatment of blast wave exposed animals with Ebselen: Figures 115 through 118 present the group (N = 22) mean results of the animals treated with Ebselen (16 mg/kg, i.p.) twice daily for 5 days following an exposure to 10, 165 dB peak SPL, free field, blast waves (11 psi shock tube charge pressure). There was a maximum PTS of approximately 49 dB at 2 kHz and 25 to 38 dB PTS at the other test

frequencies. Outer hair cell loss also peaked at 2 kHz with 85% missing cells in the 2 kHz octave band. The profile of OHC loss was very similar to that found in the original lesion calibration group and in the #2 noise only control group (see Fig. 93 and 97). Distortion product emissions were essentially eliminated from 0.8 through 4.0 kHz and severely reduced above 4.0 kHz. The cochleogram for each animal in the group is shown in Fig. 118. The results of the Ebselen treated group were qualitatively similar to the Src treated group.

(d) Statistical analysis of the results of the rescue treatments following exposure to 10, 165 dB peak SPL free field impulses (blast waves) with Ebselen, Src Inhibitor KX1-004, ALCAR, D-MET and L-NAC: Evoked potential permanent threshold shifts and inner and outer hair cell losses in octave-band lengths of the cochlea were compared among the various groups of control and drug treated animals exposed to 10, 158 dB peak SPL impulses measured at the subject's ear (11 psi shock tube charge pressure) using a two-way mixed model analysis of variance (ANOVA) with repeated measures on one factor (frequency). The probability of a type I error was set at 0.05. Statistically significant main effects of frequency were expected and found in all of the following analyses because of the frequency-specific nature of the audibility curve of the chinchilla and the noise exposure stimulus. For this reason main effects of frequency are not addressed in the following presentation of results. Post-hoc analyses (Tukey/Kramer test) were performed to establish any significant effects among pairs of the control and treatment groups.

(i) Comparison of the drug treated groups with control group #1 (see Fig. 119): There was a main effect of group for PTS and %OHC loss [(PTS: $F = 3.418$, $p = .0064$); (%OHC: $F = 2.809$, $p = .0197$)] and no main effect of group for %IHC loss ($F = .226$, $p = .9505$). The Tukey test indicated that for PTS there was no statistically significant difference between the control group and the D-MET treatment but that groups treated with either ALCAR, Ebselen, L-NAC or the Src Inhibitor all showed significantly greater PTS than the control group. For the %OHC loss there was no significant difference between the control and the ALCAR, Src Inhibitor and D-MET treated groups but that the Ebselen and L-NAC treated groups showed more OHC loss than the controls. For the IHC losses there were no significant differences among the groups. A summary bar graph of these data is shown in Fig. 119.

(ii) Comparison of the drug treated groups with control group #2 (see Fig. 120). There was no significant main effect of group for PTS, %OHC or %IHC loss [(PTS: $F = 1.439$, $p = .2153$); (IHC: $F = .953$, $p = .4497$); (%OHC: $F = 1.626$, $p = .1585$)]. The Tukey test for paired comparisons showed no significant difference between any of the treated groups and control group #2 for PTS, %IHC loss or %OHC loss. A summary bar graph of these data is shown in Fig. 120.

(iii) Comparison of the drug treated groups with the mean of control groups #1 and #2. There was a significant main effect of group for PTS ($F = 2.342$, $p = .0447$) and no significant effect of group for %IHC

or %OHC loss [(IHC: F = .494, p = .7802); (%OHC: F = 1.959, p = .0886)]. The Tukey test for paired comparisons indicated no significant difference between ALCAR, D-MET, or the Src Inhibitor and the control group for PTS and %OHC loss. There was a significant difference between both Ebselen and L-NAC treated groups and the control group with both drug treatment groups showing more PTS and %OHC loss. For %IHC loss there were no significant differences seen with the Tukey test. A summary bar graph of these data is shown in Fig. 121.

Thus, despite the variability seen among the various noise only controls and calibration groups the conclusion to be drawn from the statistics presented above is that a rescue treatment with any of the 5 drugs that were used resulted in either no beneficial effect or when there was an effect it was in the direction of exacerbating the effect of the blast wave exposure. Since there was no single drug treatment that had a significant effect on reducing the degree of hearing trauma the balance of the animals that were required by the SOW were exposed to a lower level blast wave as explained below.

The suggestion was made by a reviewer of the second annual report that we address the possibility that the damage to the cochlea may be too severe for the drugs to have any effect (i.e. the 158 dB peak SPL blast wave at the subject's ear was too intense). Phase III of the SOW called for using 180 animals run through the entire experimental protocol. One hundred and forty-three (143) animals were used to obtain the data described above. This includes the additional control (N = 20) group #2. Since we needed to run an additional 37 animals to satisfy the original SOW a series of lower level exposures and treatments were run. This additional set of exposures and treatments required 48 animals.

(e) Drug treatments following 162 dB peak free field SPL blast wave exposures. The drug administration and doses were the same as in the previous exposures.

(i) Noise only control group: Eighteen (18) animals were exposed to 10, 162 dB peak SPL free field blast waves (156 dB peak SPL at the subject's ear; 7 psi shock tube charge pressure). [Note: The mean AEP data are based on 17 animals. The AEP electrode came loose on one animal.] The group mean PTS, sensory cell loss and DPOAEs are shown in Fig. 122 through 124. The individual cochleograms for each animal in this control group are shown in Fig. 125. Maximum PTS of ~28 dB occurred at the 2 kHz test frequency with 8 to 22 dB at the other test frequencies. OHC loss of 55% also peaked at 2 kHz with progressively smaller losses through out the remainder of the cochlea. The total number of OHCs missing was 1443. The frequency specific profile of OHC loss is very similar to the calibration data shown in Fig. 11C. DPOAEs (Figs. 123 and 124) were depressed across the range of test frequencies with a maximum loss in the 1 to 2 kHz regions. The profile of DPOAE loss generally paralleled the loss of OHCs. As with all previous exposures, regardless of level, there was a wide range of sensory cell loss seen across individual animals (Fig 125).

(ii) L-NAC rescue treatment: The group mean (N = 10) results of the rescue treatment following the 156 dB blast exposure are presented in Figs. 126 through 129. PTS (Fig. 126 A and B) reached a maximum at 2 kHz of 27 dB with 10 to 24 dB PTS at the other test frequencies. OHC loss (Fig. 126 C) reached a maximum of 60% at 2 kHz with a total of 1735 missing OHCs. The largest loss of DPOAE output was seen in the 1 and 2 kHz regions. Overall the DPOAE depression (Figs. 127 and 128) was greatest in the 1 and 2 kHz region and generally paralleled the profile of OHC loss. Individual cochleograms for each animal are shown in Fig. 129. Sensory cell loss across subjects varied from minimal to very severe.

(iii) ALCAR rescue treatment: The group mean (N = 10) results of the rescue treatment with ALCAR following the 156 dB blast exposure are presented in Figs. 130 through 133. PTS (Fig. 130 A and B) reached a maximum at 2 kHz of 31 dB with 12 to 28 dB PTS at the other test frequencies. OHC loss (Fig. 130 C) reached a maximum of 57% at 1 kHz and 52% at 2 kHz with a total of 1541 missing OHCs. DPOAE depression (Figs. 131 and 132) generally paralleled the profile of OHC loss. Individual cochleograms for each animal are shown in Fig. 133.

(iv) D-MET rescue treatment: The group mean (N = 10) results of the rescue treatment with D-MET following the 156 dB blast exposure are presented in Figs. 134 through 137. [Note: The mean AEP data are based on 9 animals. The AEP electrode came loose on one animal.] PTS (Fig. 134 A and B) reached a maximum at 2 kHz of 20 dB with 4 to 18 dB PTS at the other test frequencies. OHC loss (Fig. 134 C) reached a maximum of 52% at 1 kHz and 48% at 2 kHz with a total of 1074 missing OHCs. Loss of DPOAE output was greatest in the 1 through 3 kHz regions. The DPOAE depression (Figs. 135 and 136) generally paralleled the profile of OHC loss. Individual cochleograms for each animal are shown in Fig. 137.

(f) Statistical analyses of the 156 dB blast wave exposures: The ANOVA of the data from the four 156 dB blast exposures indicated no statistically significant difference among the groups in PTS, %OHC or %IHC loss [(PTS: $F = 0.837$, $p = 0.4816$); (%IHC: $F = 1.001$, $p = 0.4023$); (%OHC: $F = 0.538$, $p = 0.6589$)]. Pair wise comparisons using the Tukey test indicated significantly greater PTS in the ALCAR treated group than in the D-MET group. There were also differences in IHC loss between the control and L-NAC groups and between D-MET and L-NAC groups, with L-NAC showing the greatest IHC loss. There were no differences in OHC loss among the groups. Summary bar graphs of these data are shown in Fig. 138.

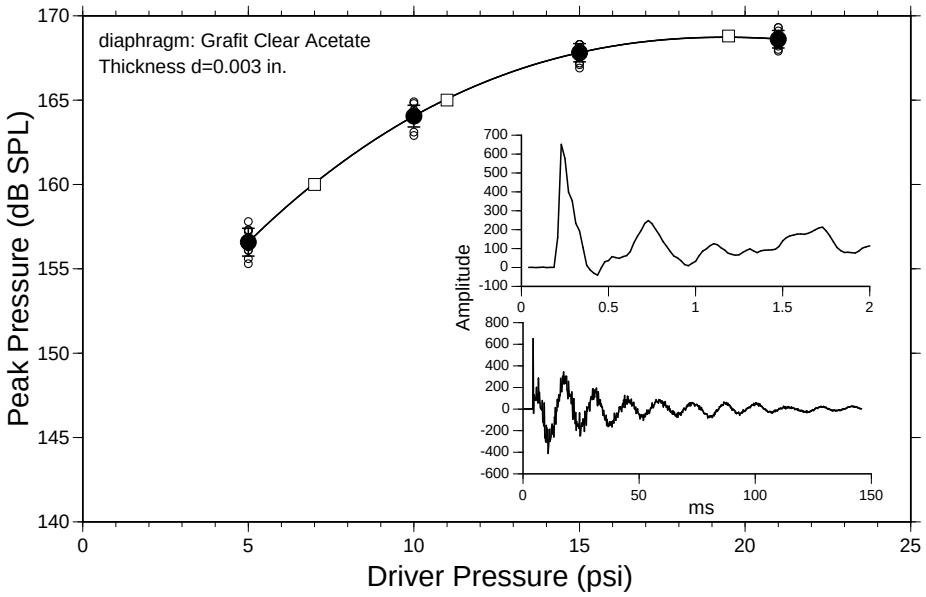


Figure 2. Peak pressure of the incident shock wave at the entrance of the 36-inch reverberant container. Inset: an example of the pressure-time history of the impulse. Amplitude is in arbitrary units. (N=10 shots at each driver pressure). The measurements were made with an 1/8" B&K condenser microphone oriented at a grazing incidence to the normal shock. □ = driver pressure and peak SPL used to produce the three exposure conditions for the Phase I lesion calibration.

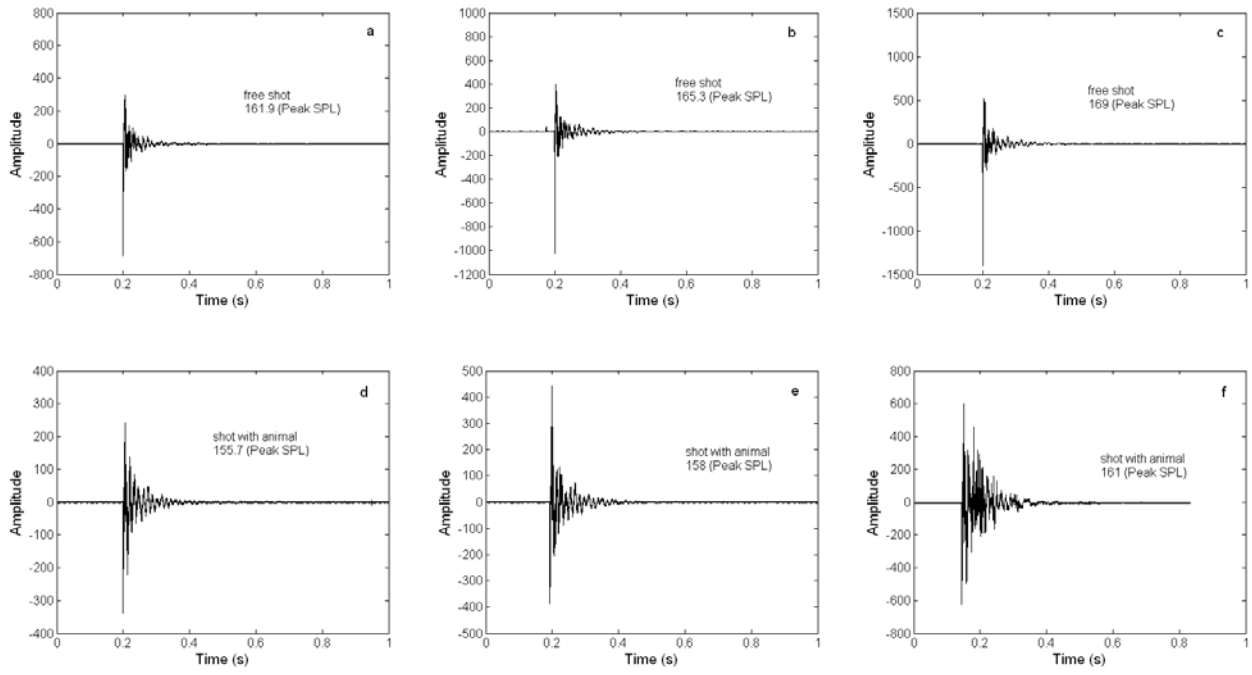


Figure 3. Examples of the impulse waveform measured in the free field (Panel a, b & c) and with the animal present and oriented with the right ear in the downstream position (Panel d, e & f). Note the greater reverberant character of the impulse in panels d, e and f. Panel a & d driver pressure = 7 psi, free field peak SPL = 162 dB; at the animal's right ear, peak SPL = 156 dB. Panel b & e driver pressure = 11 psi, free field peak SPL = 165 dB; at the animal's right ear, peak SPL = 158 dB. Panel c & f driver pressure = 19-20 psi, free field peak SPL = 169 dB; at the animal's right ear, peak SPL = 161 dB. Note: different amplitude scale in arbitrary units.

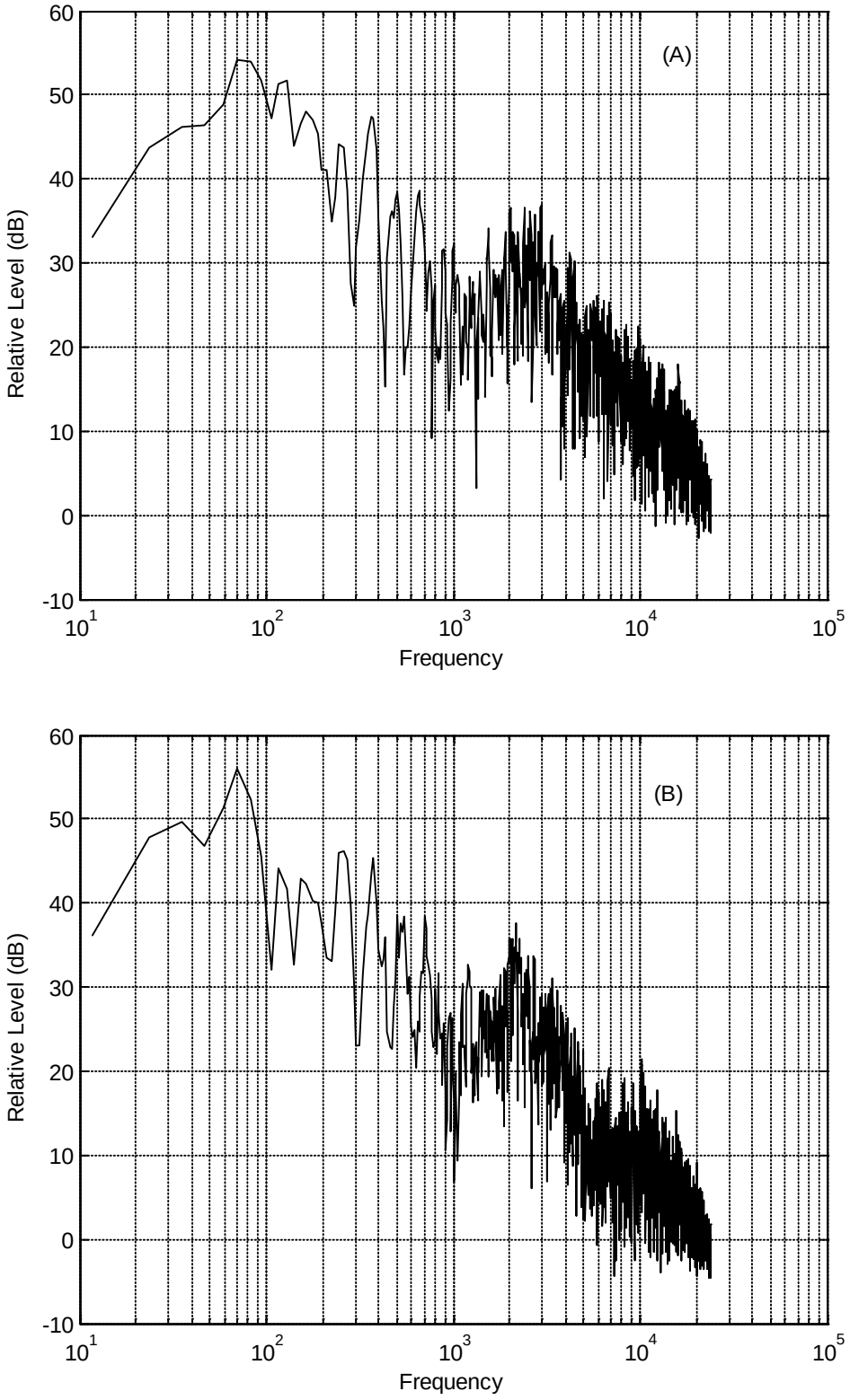


Figure 4. The relative spectral level of the noise impulse (blast wave) produced by the shock tube operated at an 11 psi driver pressure. (A) Measured in the free field where the peak SPL was 165 dB. (B) Measured at the experimental ear of an animal during an exposure; peak SPL = 158 dB.

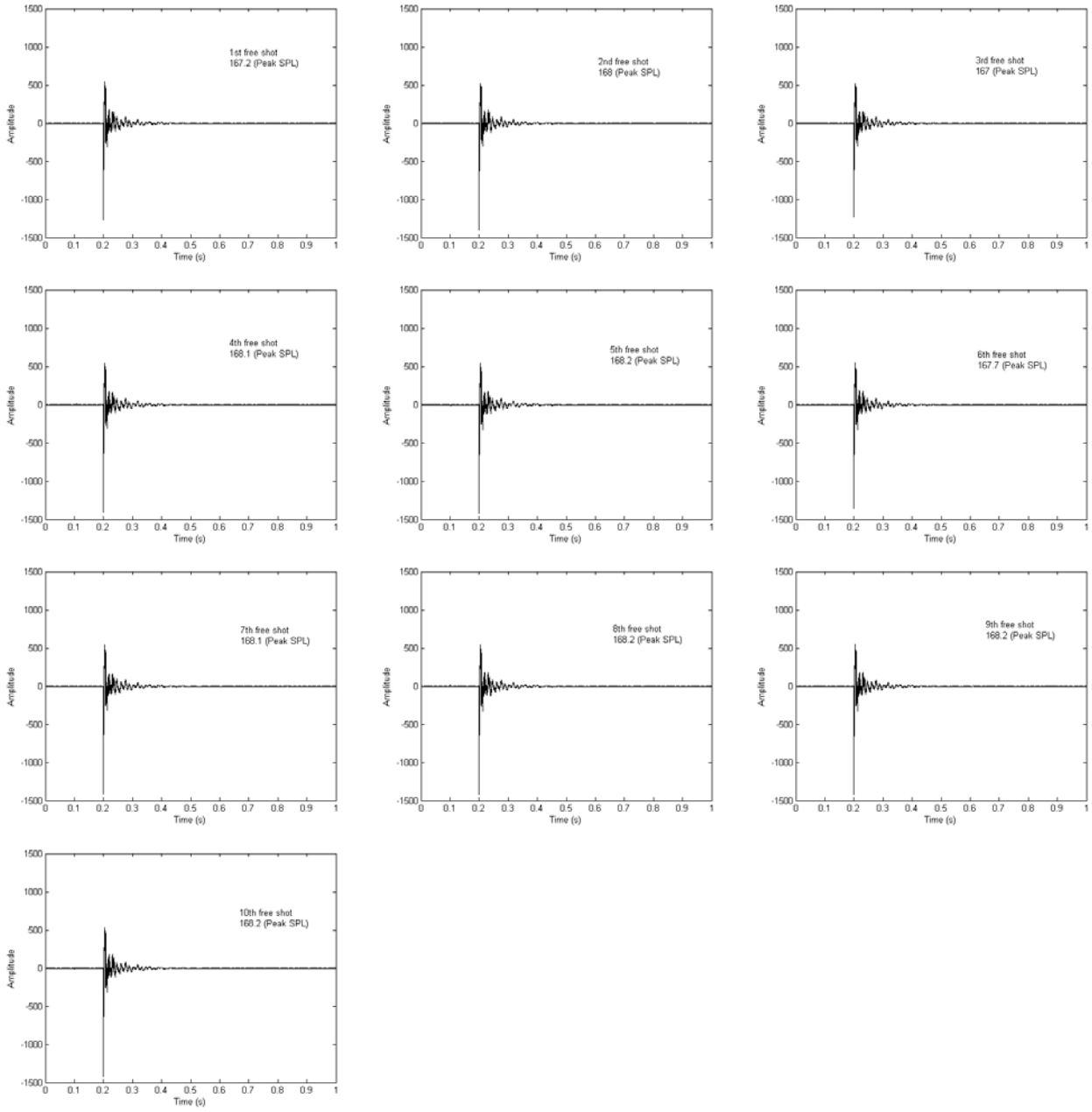


Figure 5. The pressure-time waveforms from 10 shock tube discharges. Discharge pressure = 19-20 psi. Diaphragm thickness = 0.003 inch Grafit clear acetate. Measurements were made using the 1/8” B&K condenser microphone at the location of the animal’s head. Animal was not present. The microphone was at a grazing incidence to the advancing normal shock front. Mean peak SPL = 168 dB.

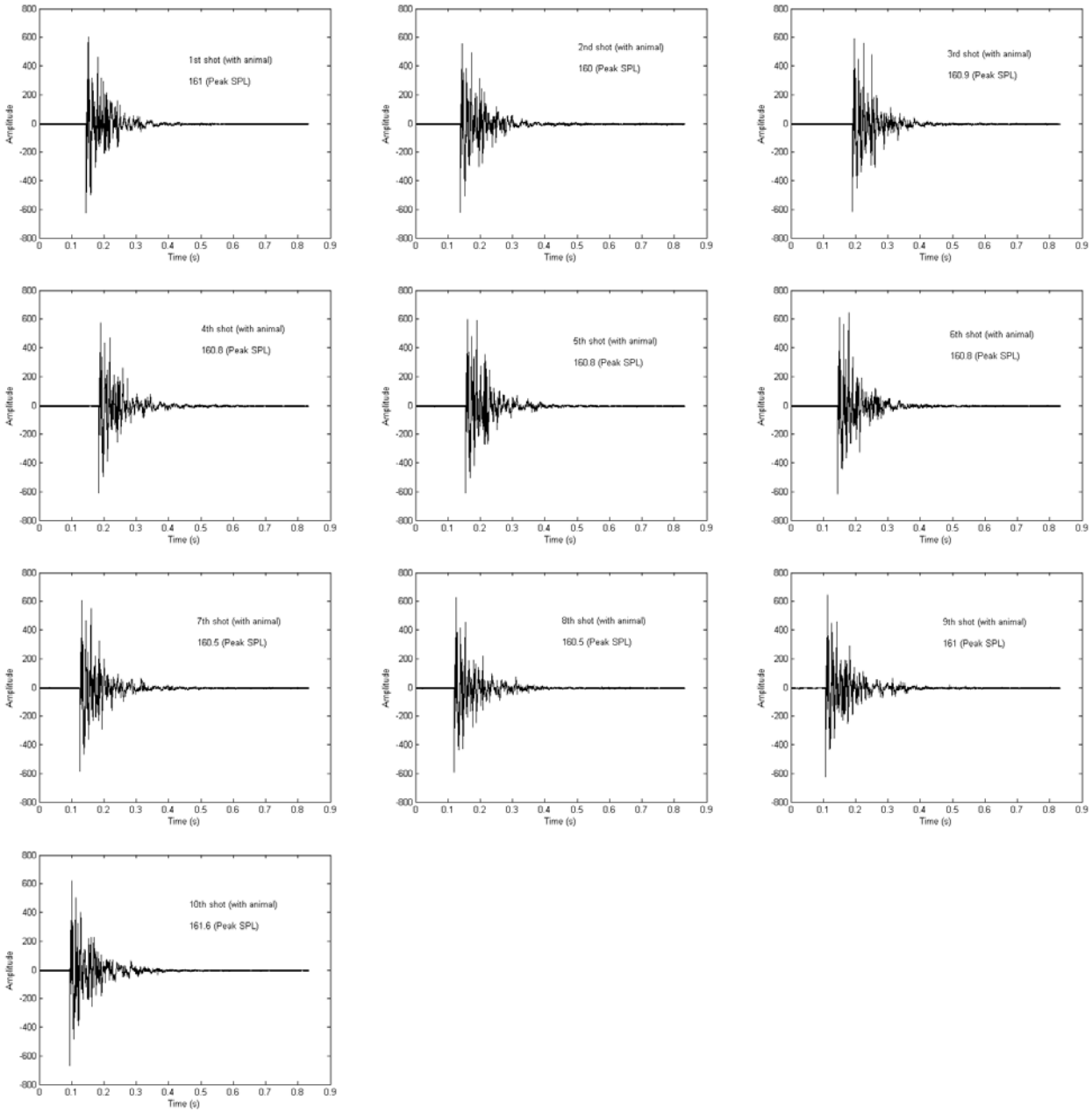


Figure 6. The pressure-time waveforms resulting from 10 shock tube discharges. Shock tube discharge pressure = 19-20 psi. Diaphragm thickness is 0.003 inch of Grafit clear acetate. Measurements were made using the 1/8" B&K condenser microphone at the down stream ear with the animal in place. The microphone was at a grazing incidence to the advancing normal shock front. Mean peak SPL = 160.8 dB.

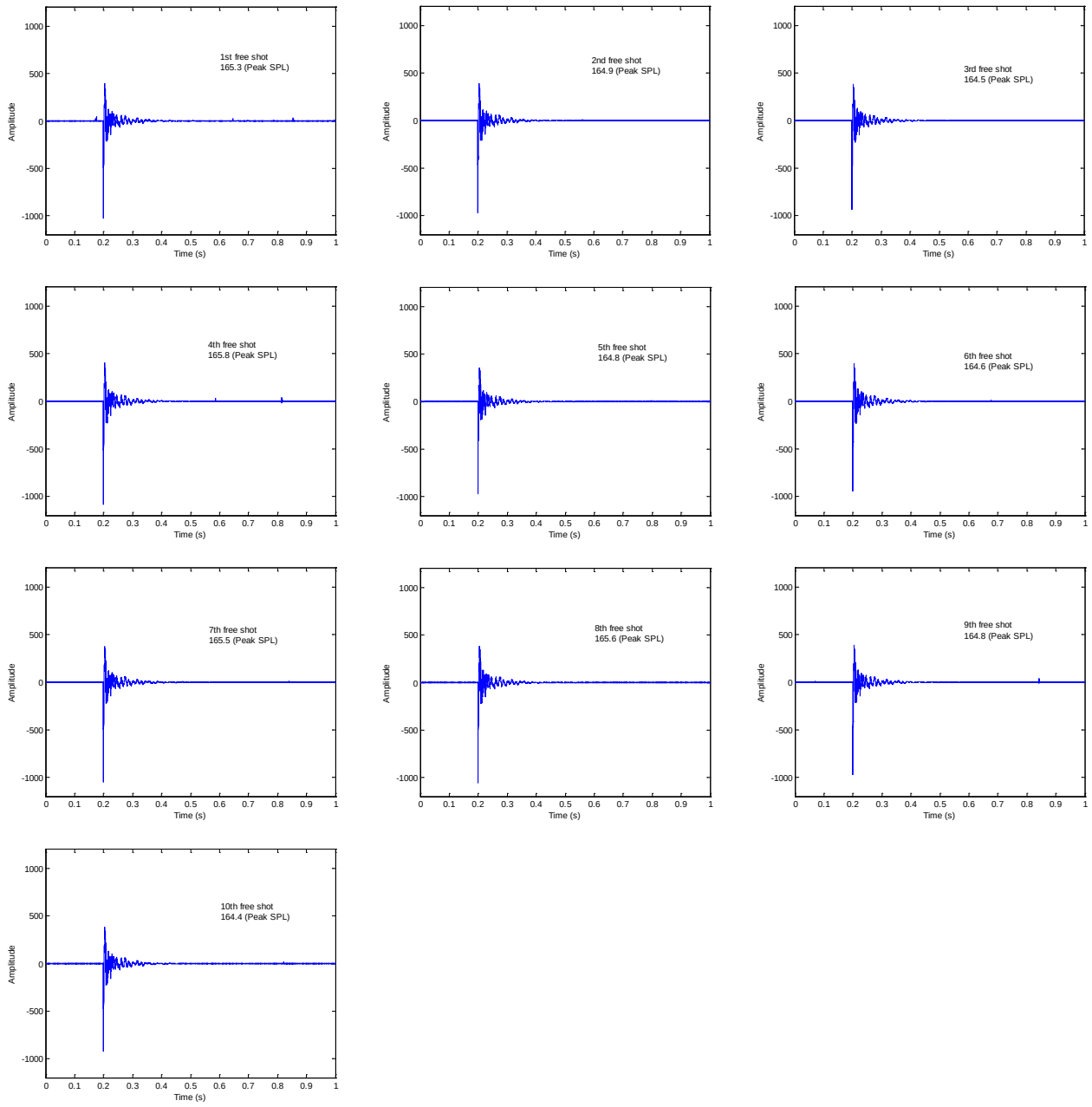


Figure 7. The pressure-time waveforms from 10 shock tube discharges. Discharge pressure = 11 psi. Diaphragm thickness = 0.003 inch Grafit clear acetate. Measurements were made using the 1/8” B&K condenser microphone at the location of the animal’s head. Animal was not present. The microphone was at a grazing incidence to the advancing normal shock front. Mean peak SPL = 165 dB.

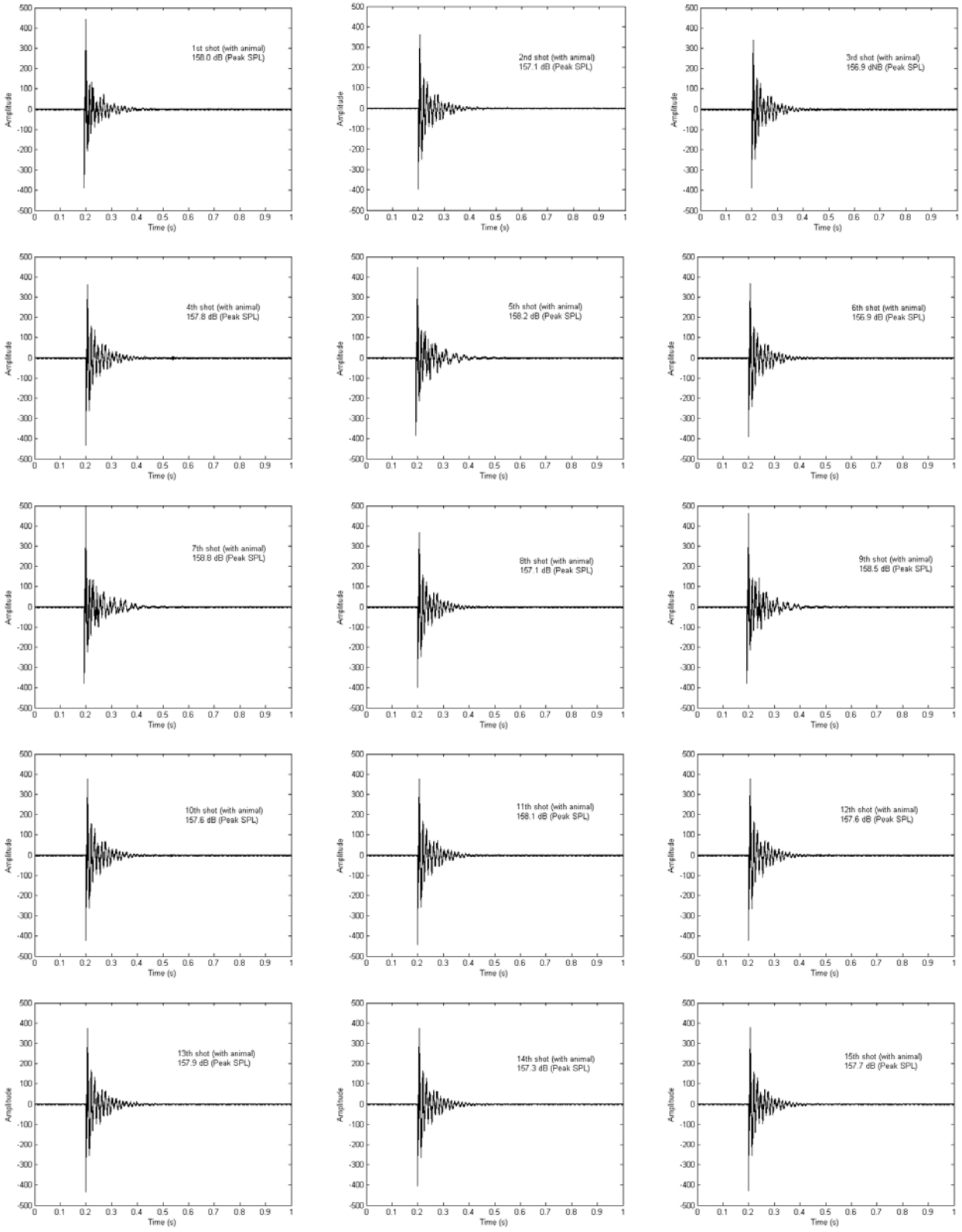


Figure 8. The pressure-time waveforms resulting from 15 shock tube discharges. Shock tube discharge pressure = 11 psi. Diaphragm thickness is 0.003 inch of Grafit clear acetate. Measurements were made using the 1/8" B&K condenser microphone at the down stream ear with the animal in place. The microphone was at a grazing incidence to the advancing normal shock front. Mean peak SPL = 157.7 dB.

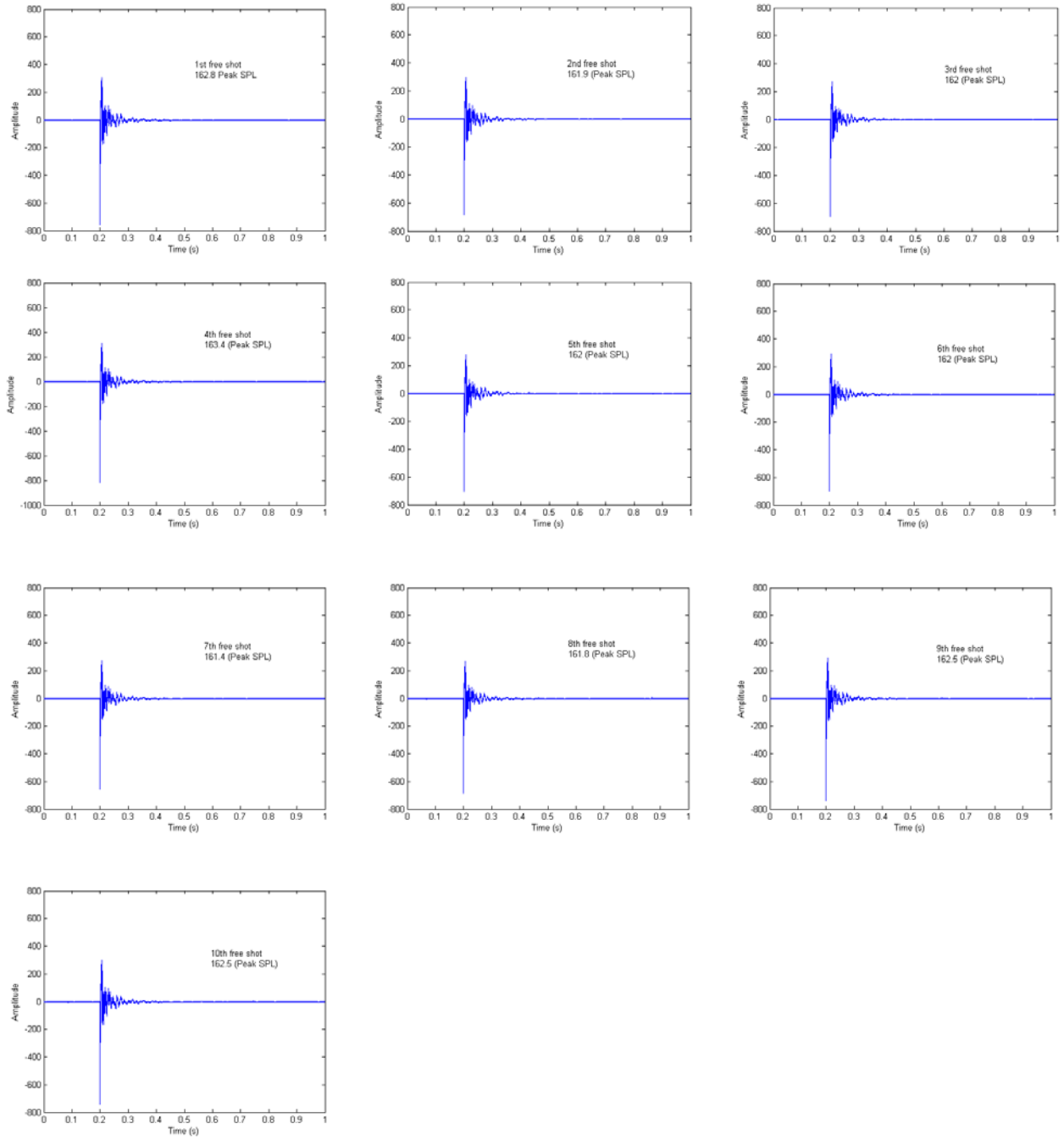


Figure 9. The pressure-time waveforms from 10 shock tube discharges. Discharge pressure = 7 psi. Diaphragm thickness = 0.003 inch Grafit clear acetate. Measurements were made using the 1/8" B&K condenser microphone at the location of the animal's head. Animal was not present. The microphone was at a grazing incidence to the advancing normal shock front. Mean peak SPL = 162.2 dB.

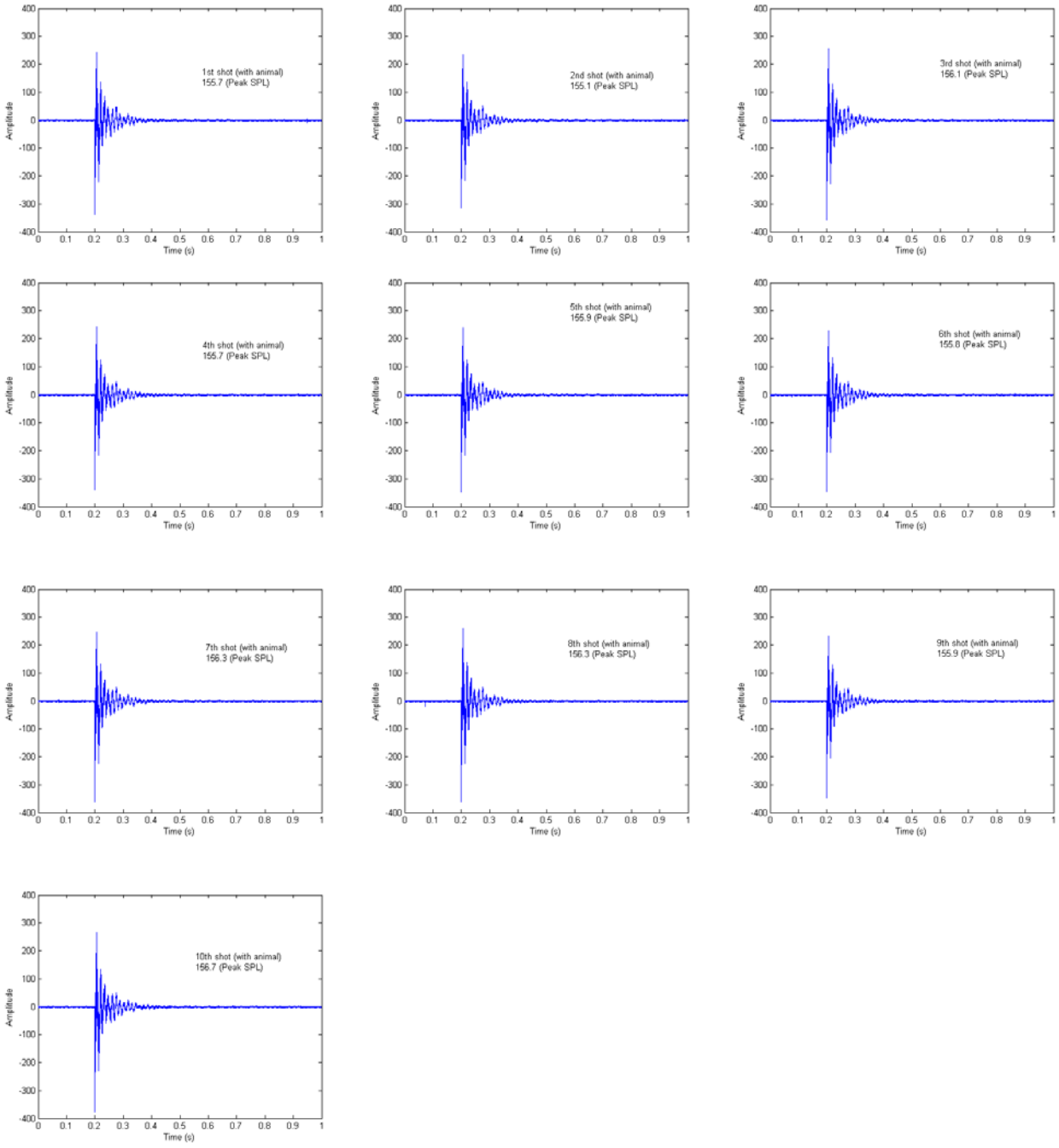


Figure 10. The pressure-time waveforms resulting from 10 shock tube discharges. Shock tube discharge pressure = 7 psi. Diaphragm thickness is 0.003 inch of Grafit clear acetate. Measurements were made using the 1/8" B&K condenser microphone at the down stream ear with the animal in place. The microphone was at a grazing incidence to the advancing normal shock front. Mean peak SPL = 156 dB.

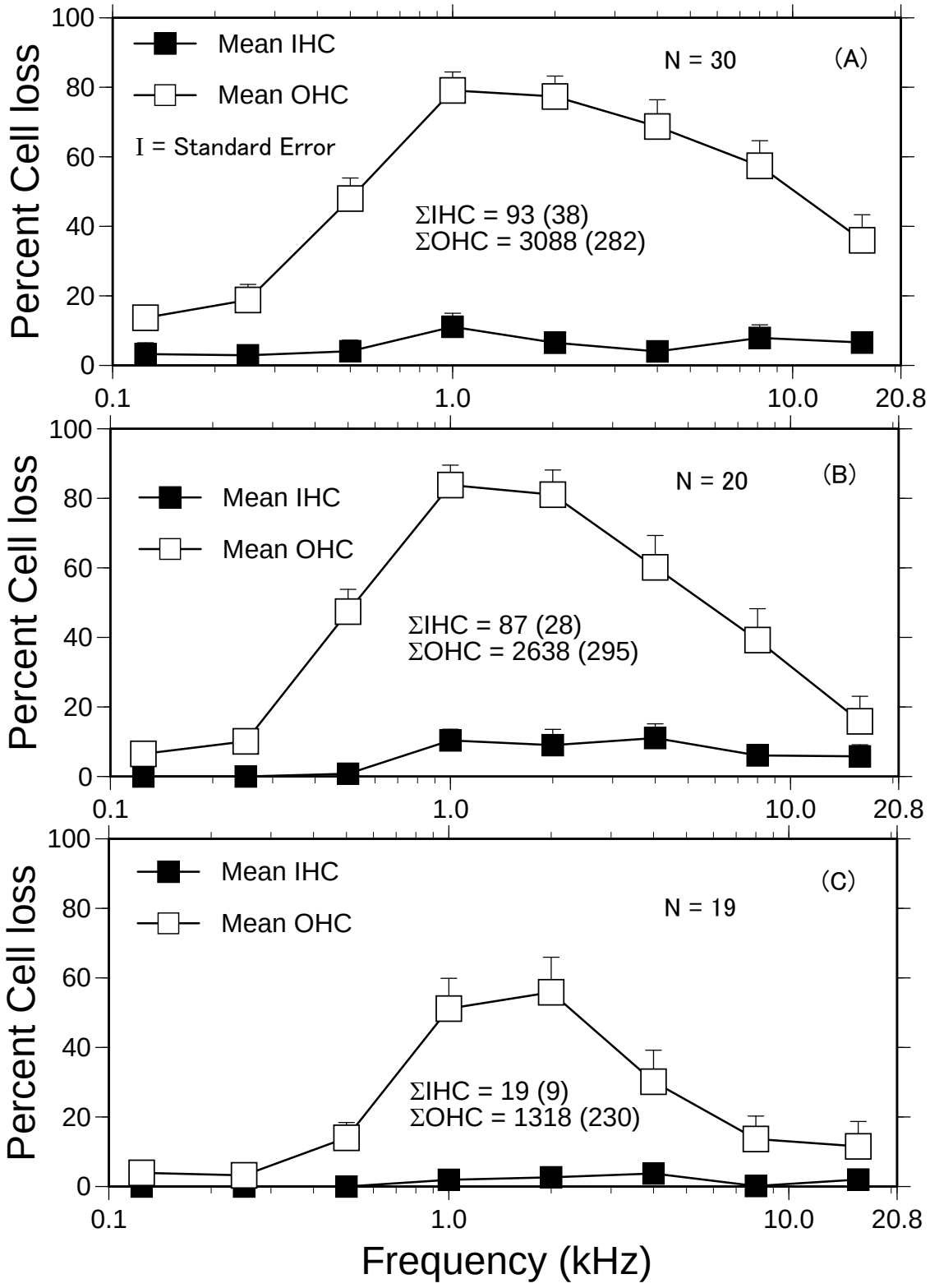


Figure 11. Phase I lesion calibration: The group mean percent inner and outer hair cell (IHC, OHC) loss for chinchillas exposed to 10 impulses at (A) 161 dB peak SPL, (B) 158 dB peak SPL and (C) 156 dB peak SPL measured at the experimental ear with the animal in place. Each data point represents the group mean cell loss estimated over a one octave band length of basilar membrane centered at the indicated frequencies. Animals were euthanized 30 days post exposure. ΣIHC & ΣOHC = total mean number of inner and outer hair cells missing. (T = standard error).

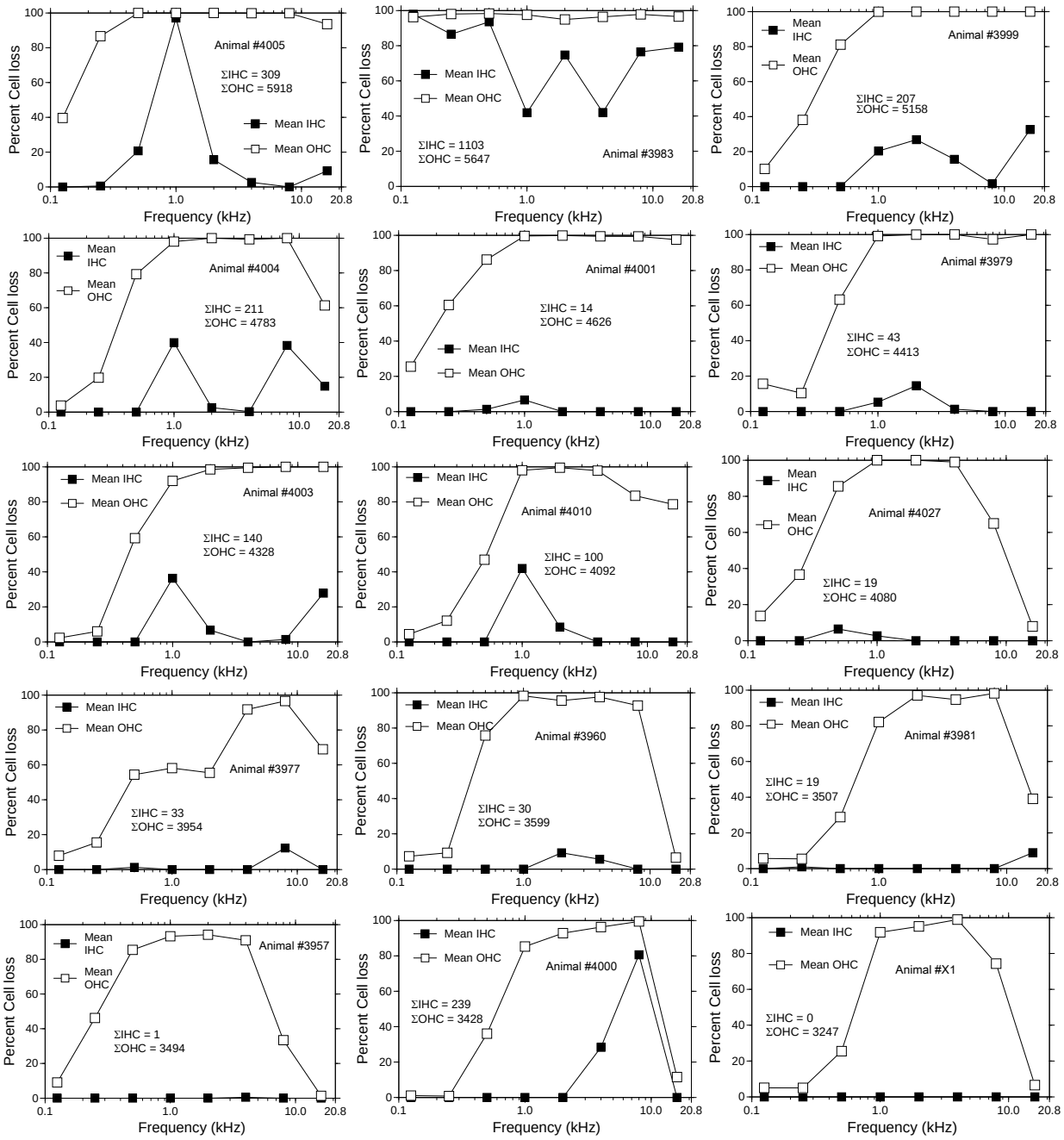


Figure 12. Individual cochleograms for the 30 chinchillas exposed to 10 shock waves over ~1.2 minutes at a peak SPL of 161 dB (19-20 psi driver pressure). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure.

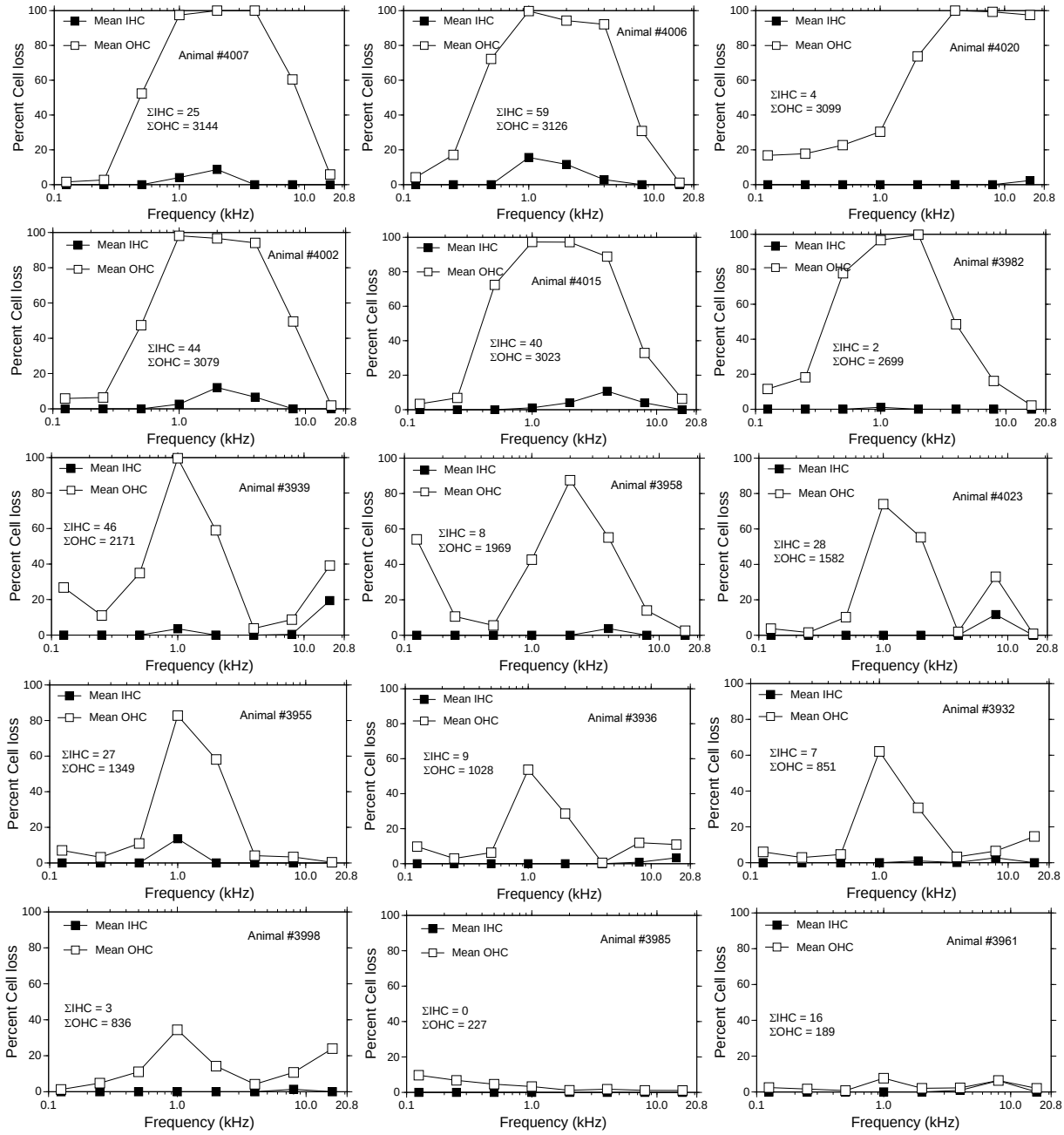


Figure 12 (con't). Individual cochleograms for the 30 chinchillas exposed to 10 shock waves over ~1.2 minutes at a peak SPL of 161 dB (19-20 psi driver pressure). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure.

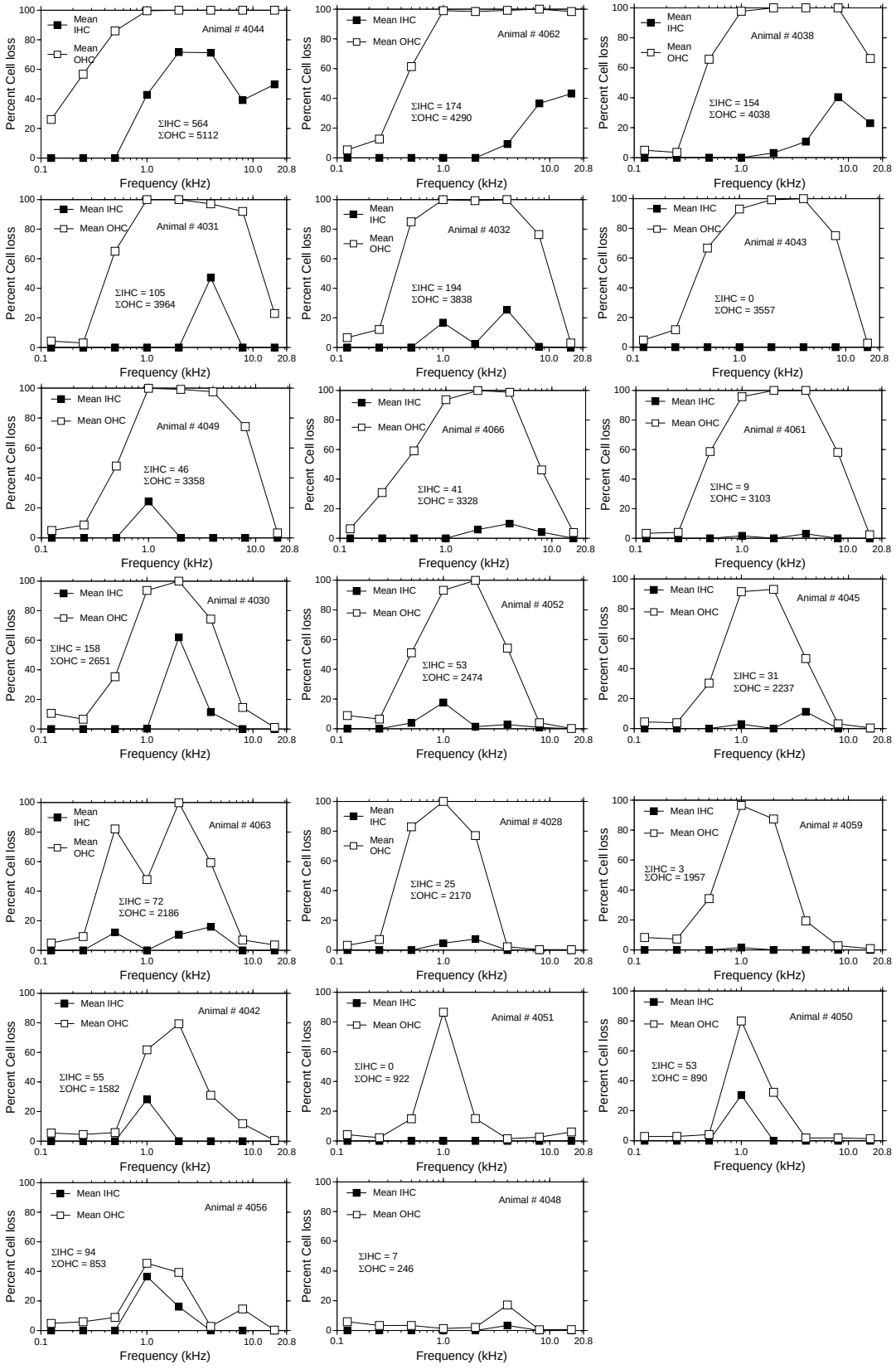


Figure 13. Individual cochleograms for the 20 chinchillas exposed to 10 shock waves over ~1.2 minutes at a peak SPL of 158 dB (11 psi driver pressure). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure.

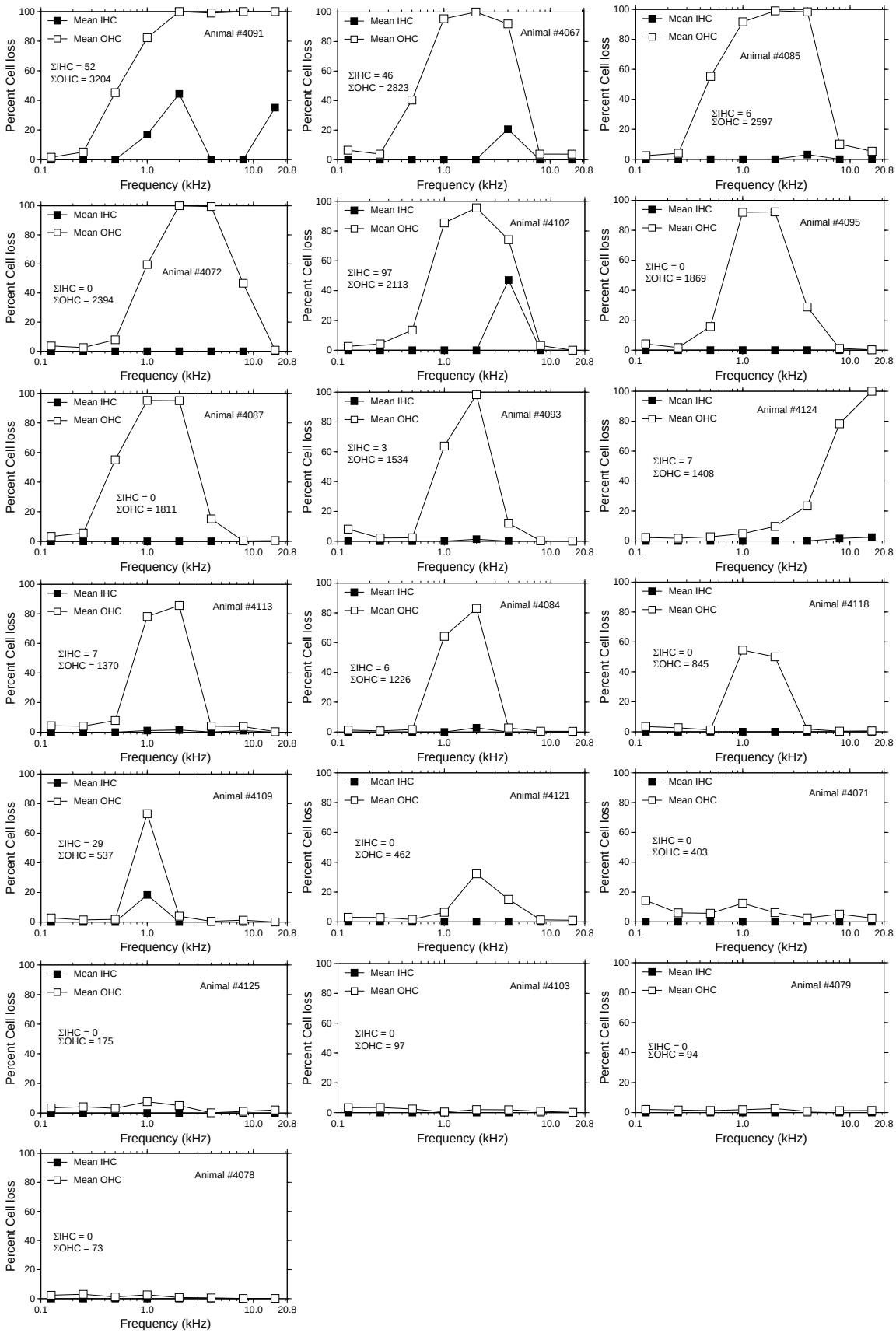


Figure 14. Individual cochleograms for the 19 chinchillas exposed to 10 shock waves over ~1.2 minutes at a peak SPL of 156 dB (7 psi driver pressure). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure.

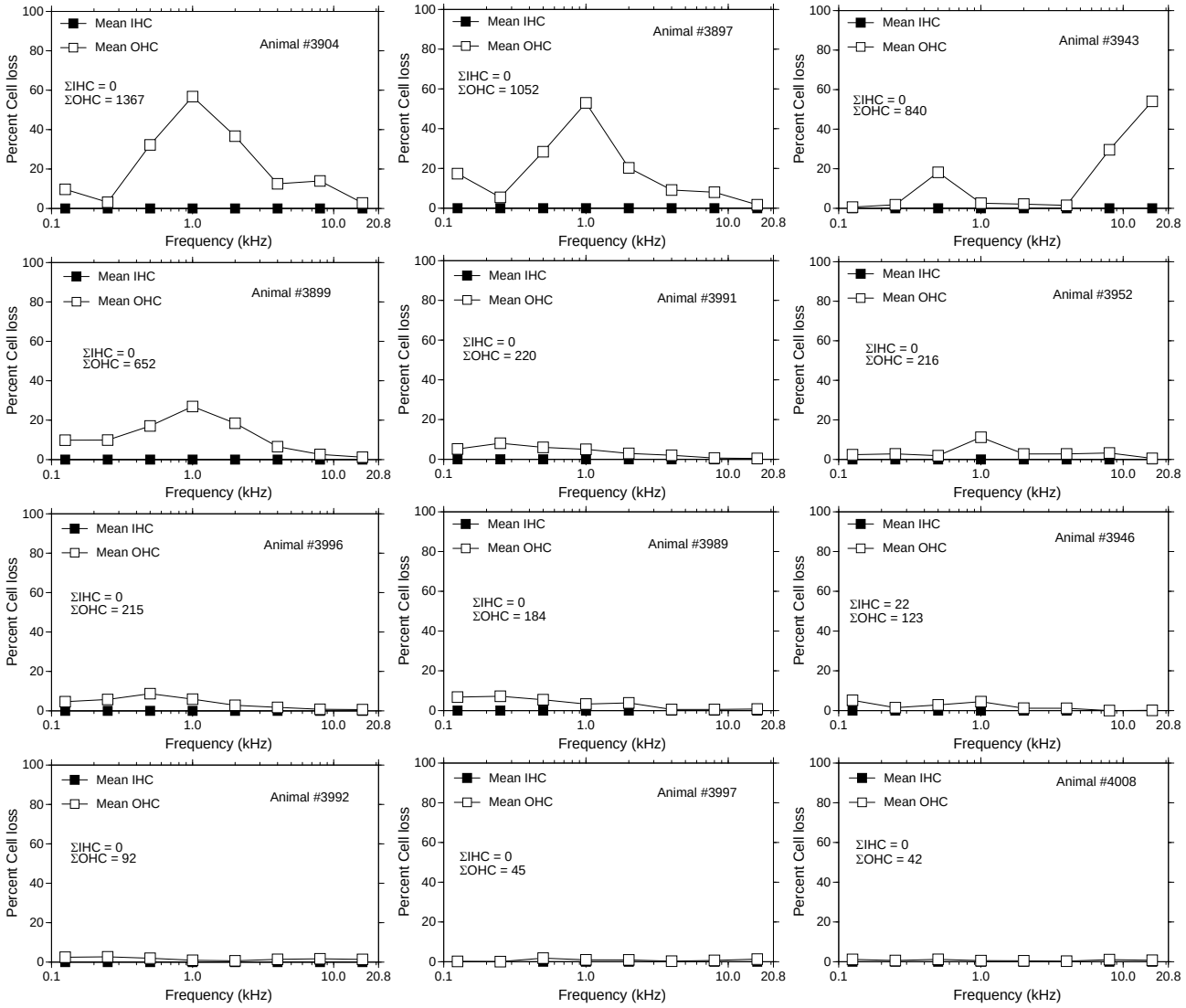


Figure 15. Individual cochleograms for the 12 chinchillas that were euthanized immediately following exposure to 10 shock waves over ~1.2 minutes at a peak SPL of 161 dB (19-20 psi driver pressure). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost.

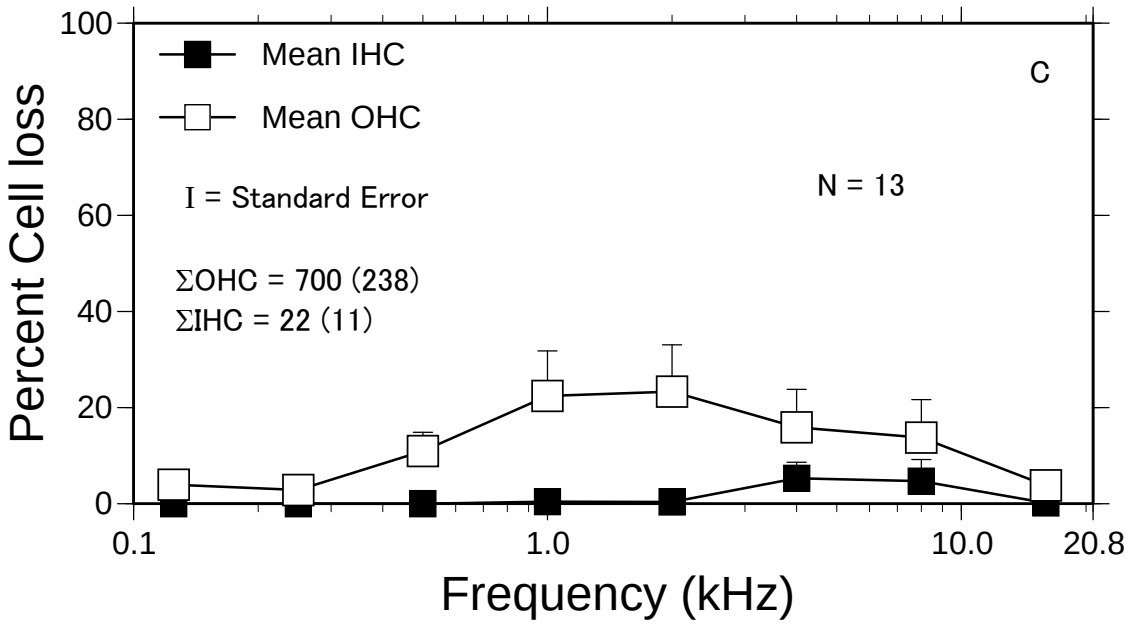


Figure 16. The group mean (N=13) cochleogram for the animals exposed to 10 impulses of the 158 dB peak SPL shock wave (11 psi charge pressure) that were also subjected to pre and immediate post exposure tympanometry (see Table 5). The animals were exposed with the experimental ear in the down stream position. (T = standard error). Animals were euthanized 30d postexposure.

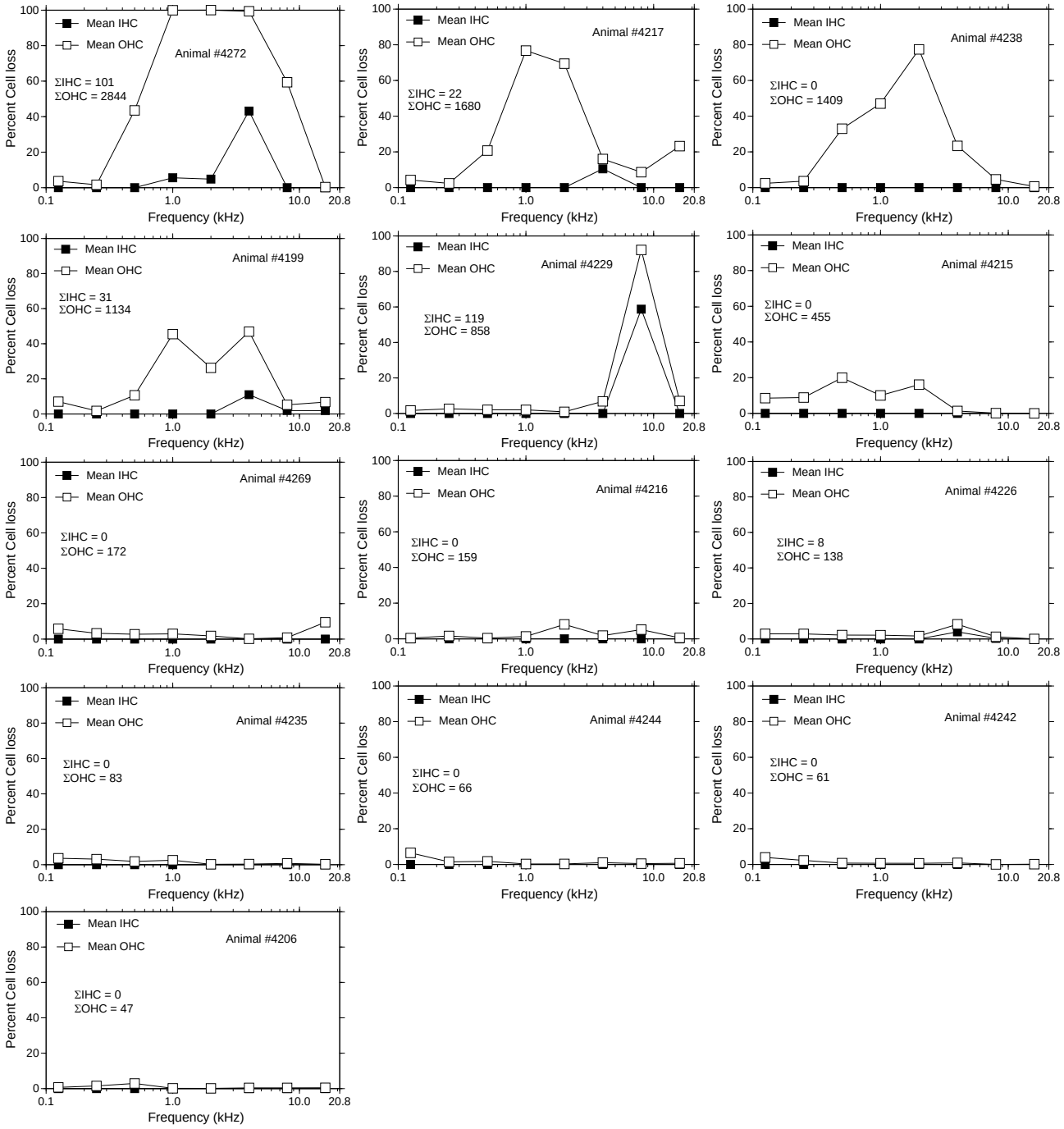
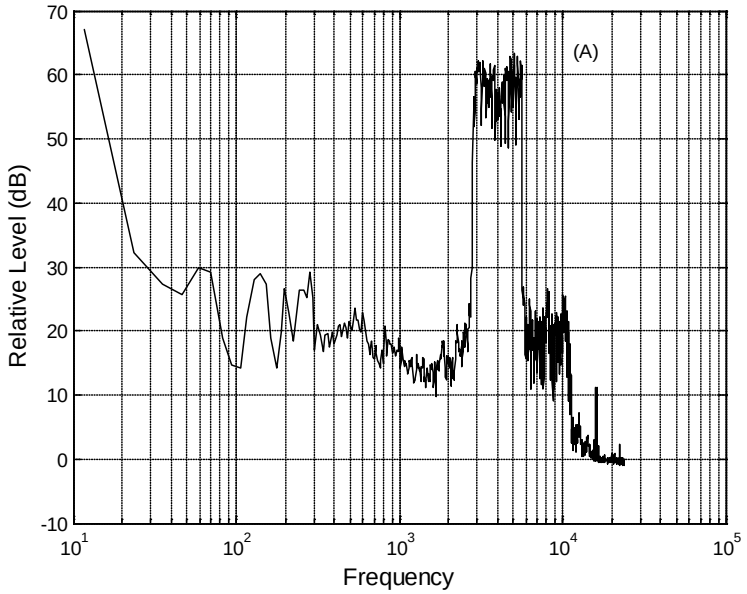
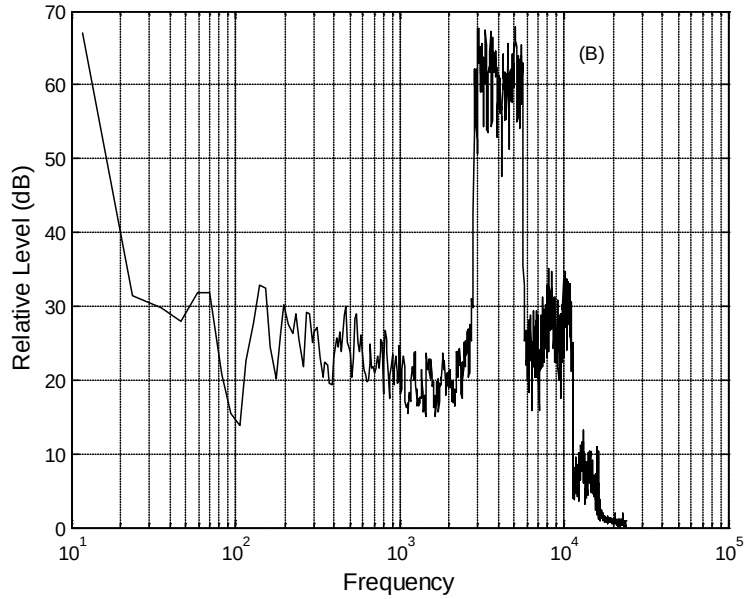


Figure 17. Individual cochleograms for the 13 animals exposed to 10 impulses of the 158 dB peak shock wave (11 psi charge pressure) that were subjected to pre and post exposure tympanometry. Cochleograms are arranged in order of decreasing outer hair cell (OHC) loss.



105.7 dB (Cage #4)	104.7 dB (Cage #3)
105.5dB (Cage #1)	104.6 dB (Cage #2)

Overall mean level: 105.1 dB SPL



108.0 dB (Cage #4)	107.9 dB (Cage #3)
108.2dB (Cage #1)	108.3 dB (Cage #2)

Overall mean level: 108.1 dB SPL

Figure 18. The relative spectral level and the noise levels measured in the center of the individual cages for the (A) 105 dB and (B) 108 dB SPL, 4 kHz octave band of noise. Individual cage dimensions: L = 11; H = 6; W = 6 inches.

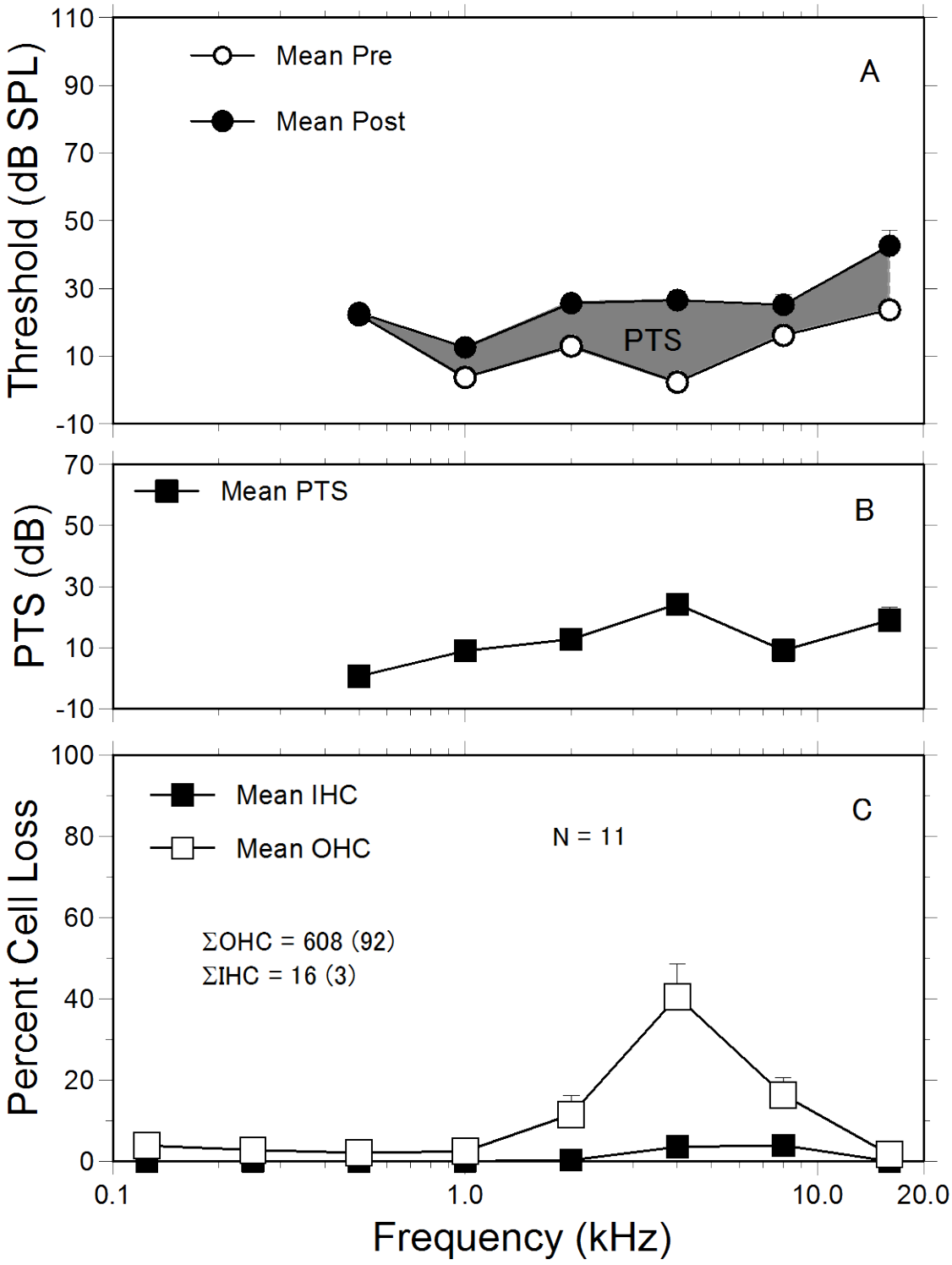


Figure 19. Phase II: Noise only control group (N = 11) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. (A) Pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) Percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

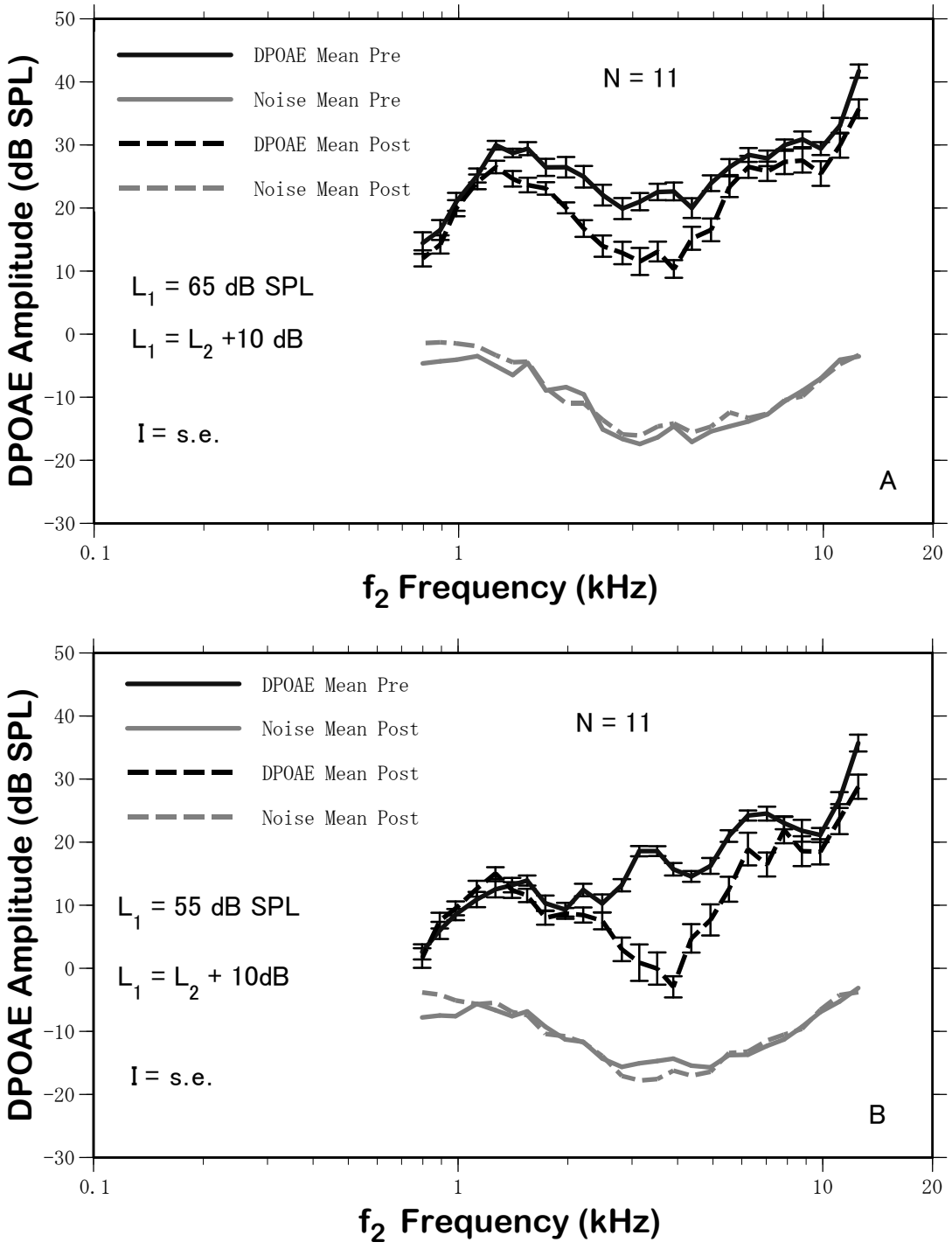


Figure 20. Phase II: Noise only control group mean (N = 11) pre and post exposure cubic ($2f_1$- f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

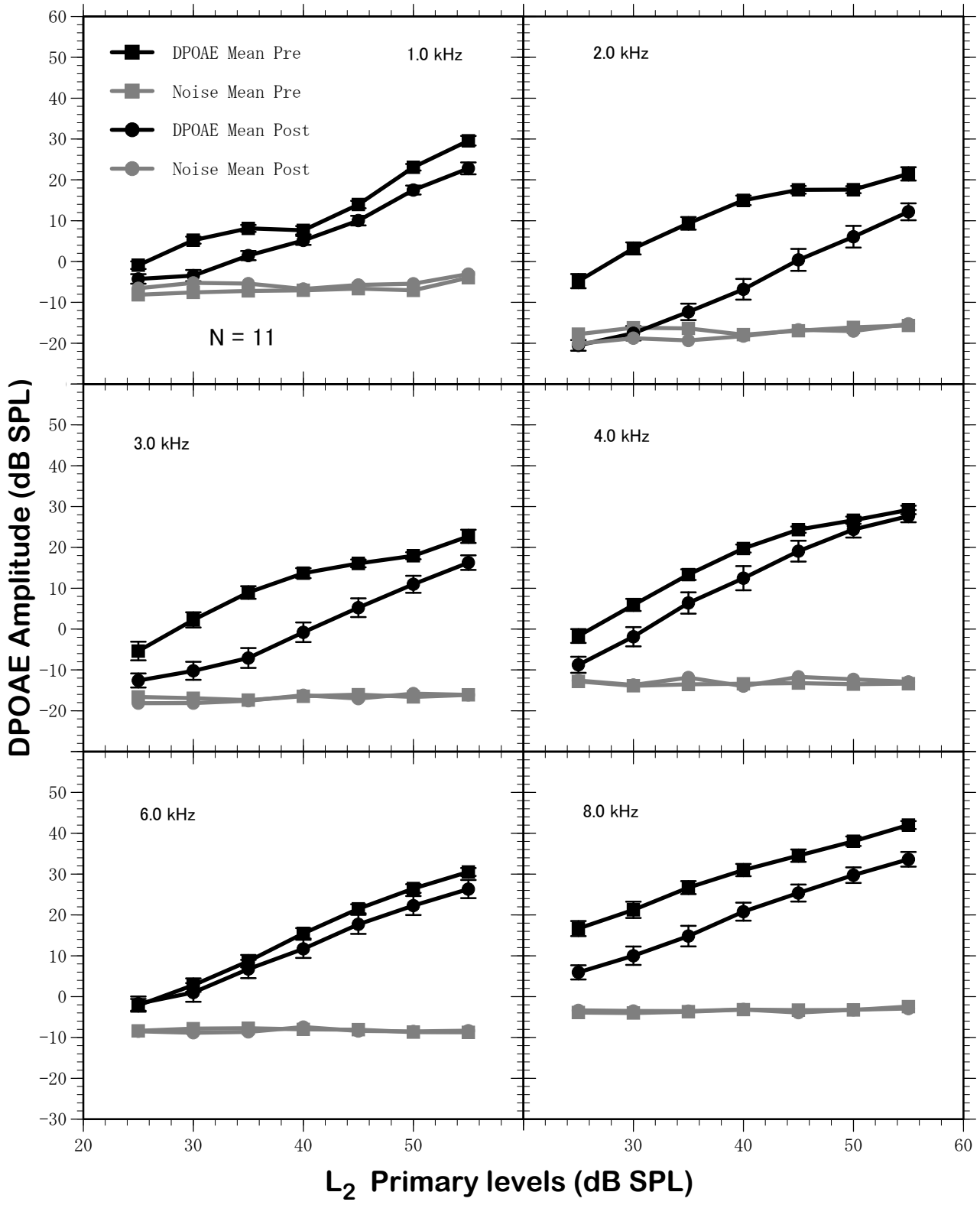


Figure 21. Phase II: Noise only control group mean (N=11) pre and post exposure cubic ($2f_1$- f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

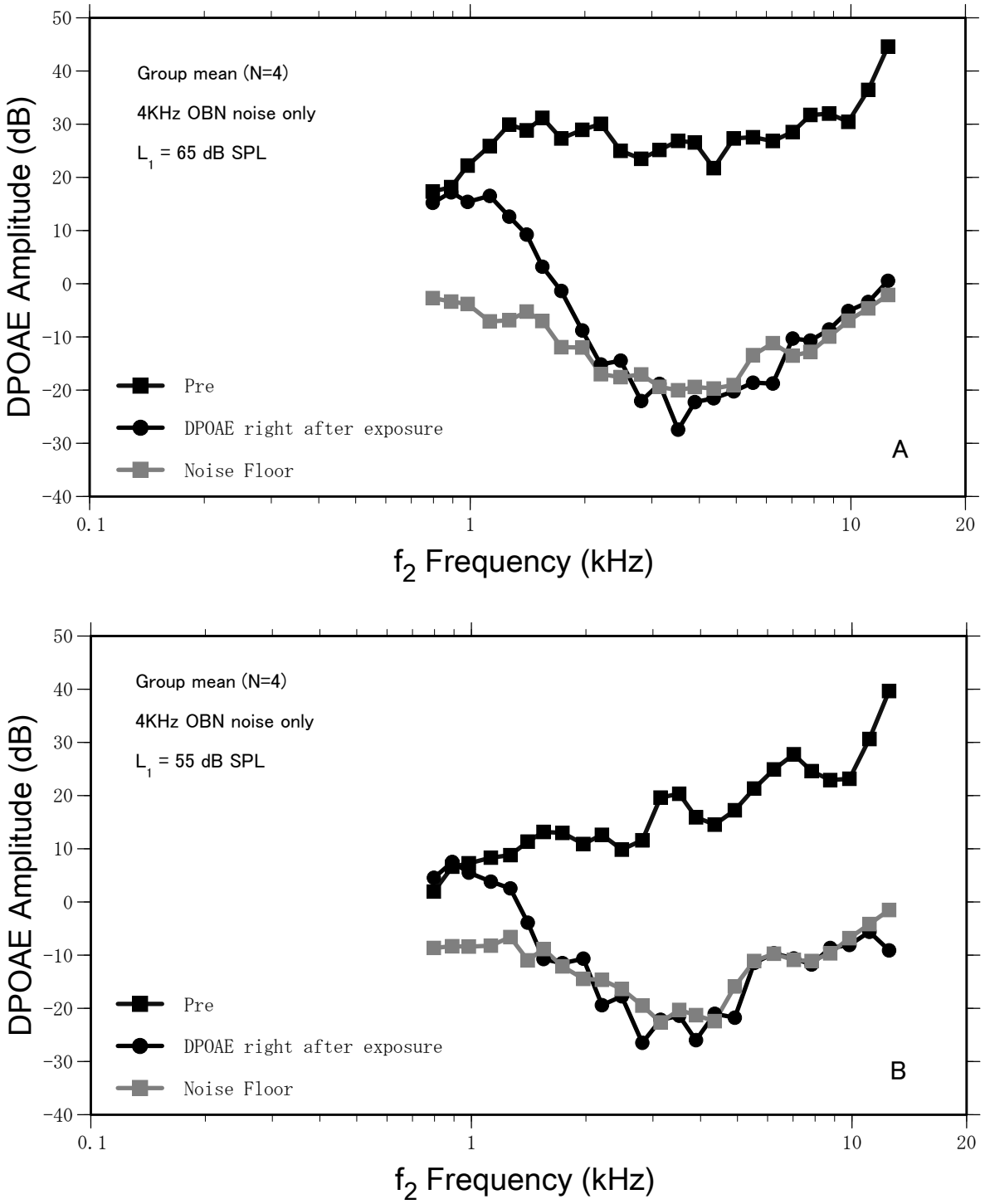


Figure 22. The mean (N = 4) pre and immediate postexposure cubic ($2f_1$- f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency for the group exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 h. The upper and lower primary frequencies f_1 and f_2 have levels L_1 and L_2 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$.

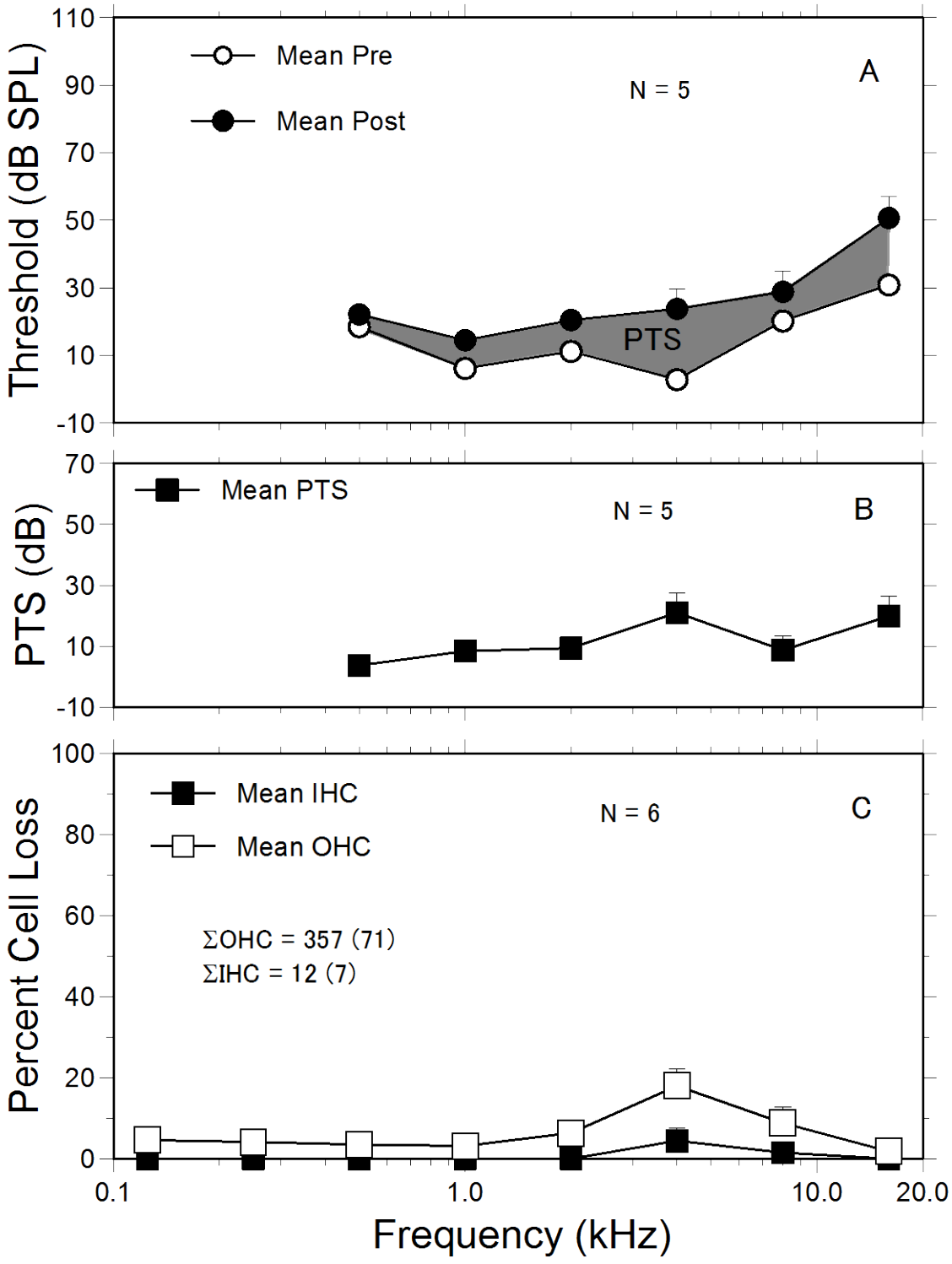


Figure 23. Phase II: Noise only control group (N = 6, reconfigured exposure set up) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

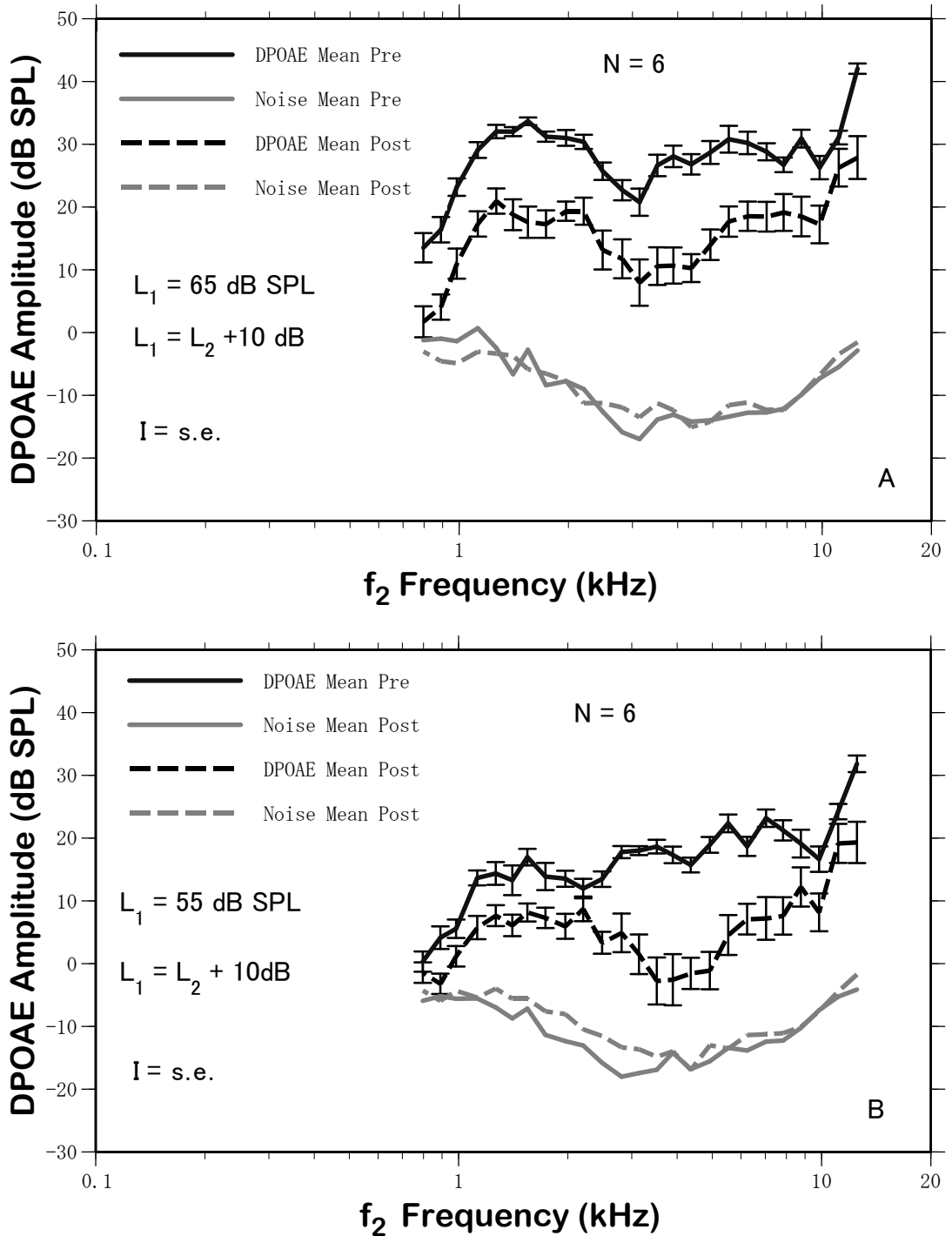


Figure 24. Phase II: Noise only control group mean (N = 6, reconfigured exposure set up) pre and post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

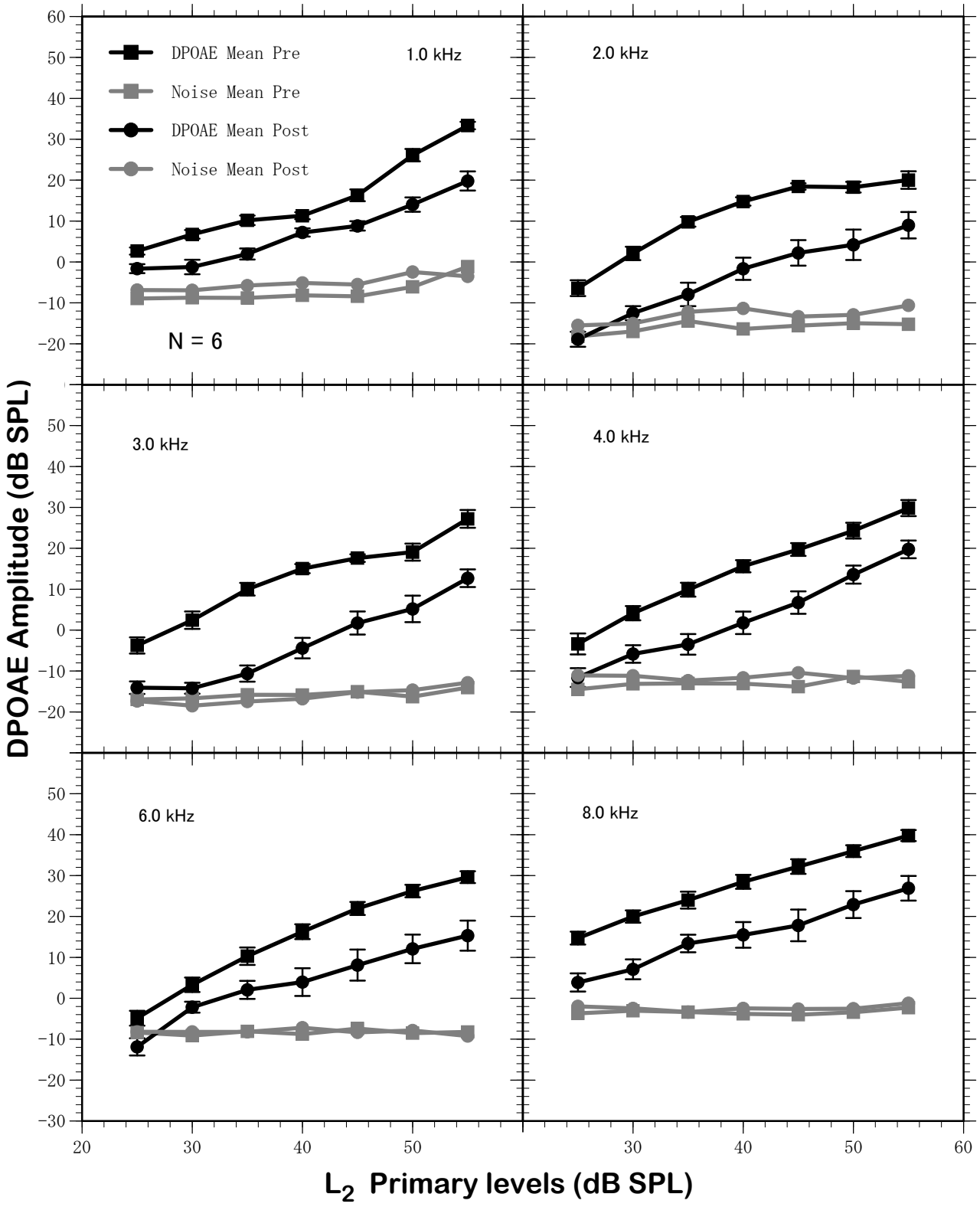


Figure 25. Phase II: Noise only control group mean (N = 6, reconfigured exposure set up) pre and post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

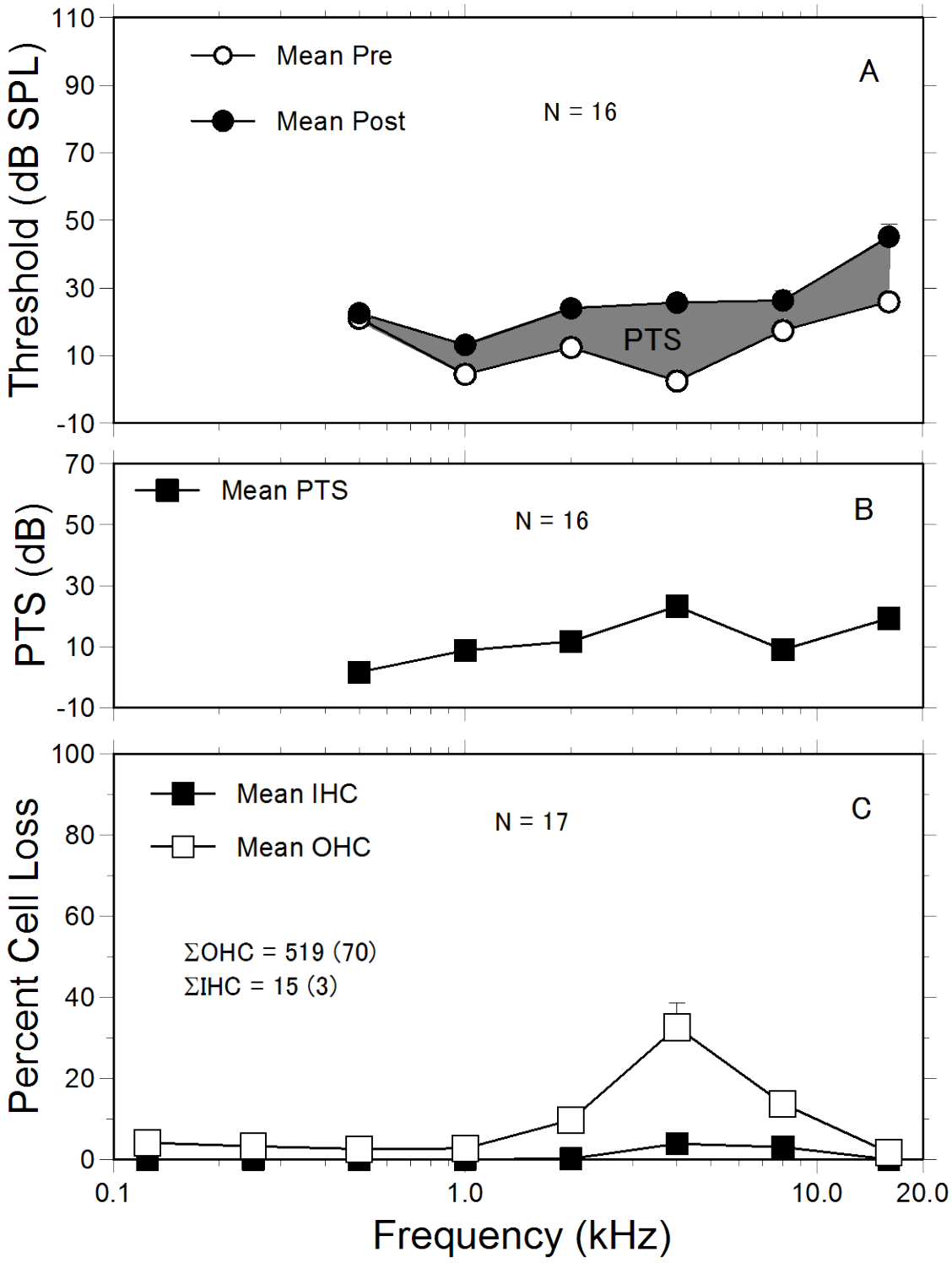


Figure 26. Phase II: Noise only combined control group (N=17) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parantheses. (T = standard error).

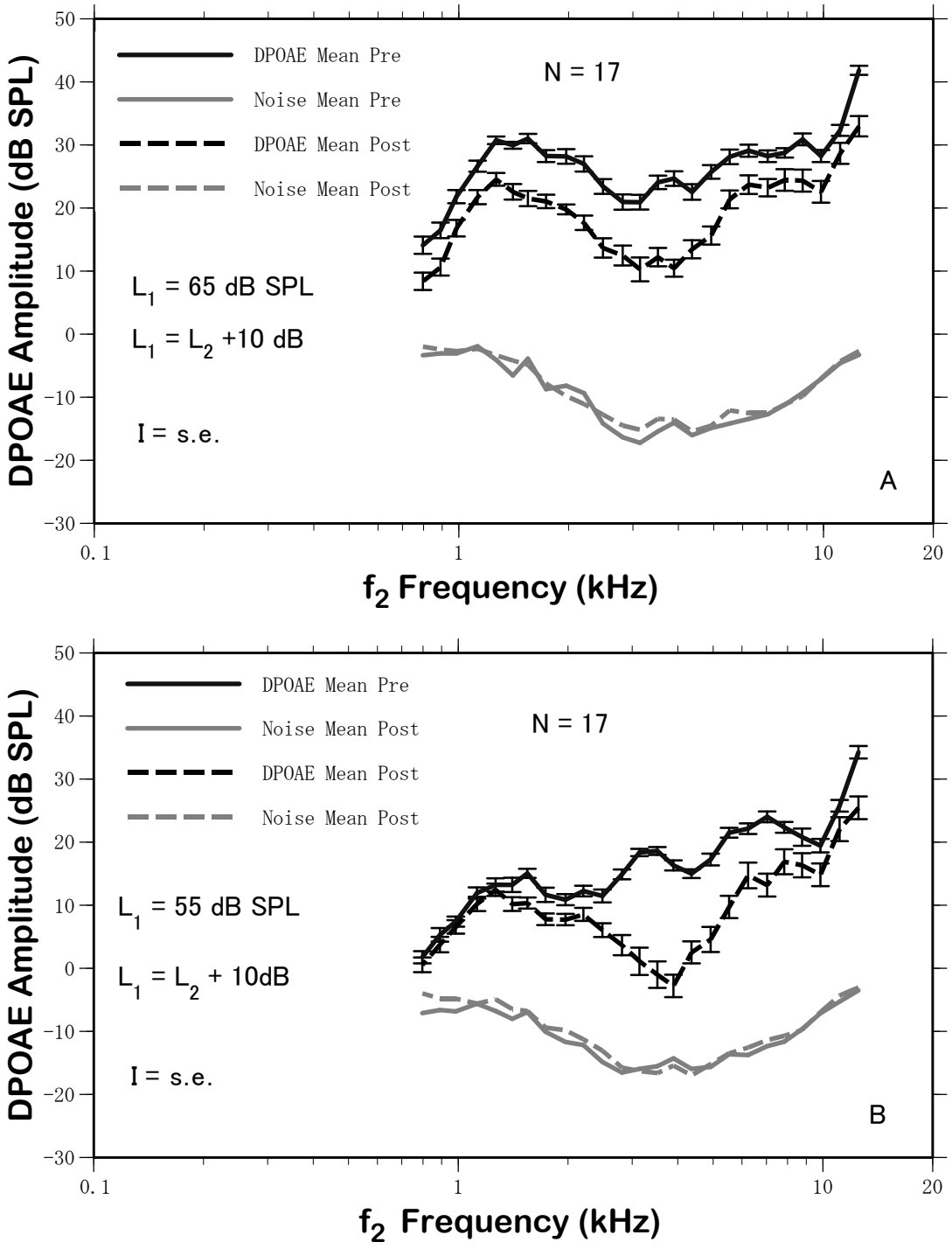


Figure 27. Phase II: Noise only combined control group mean (N=17) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency for the combined noise only control group. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where L_1 = L_2+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

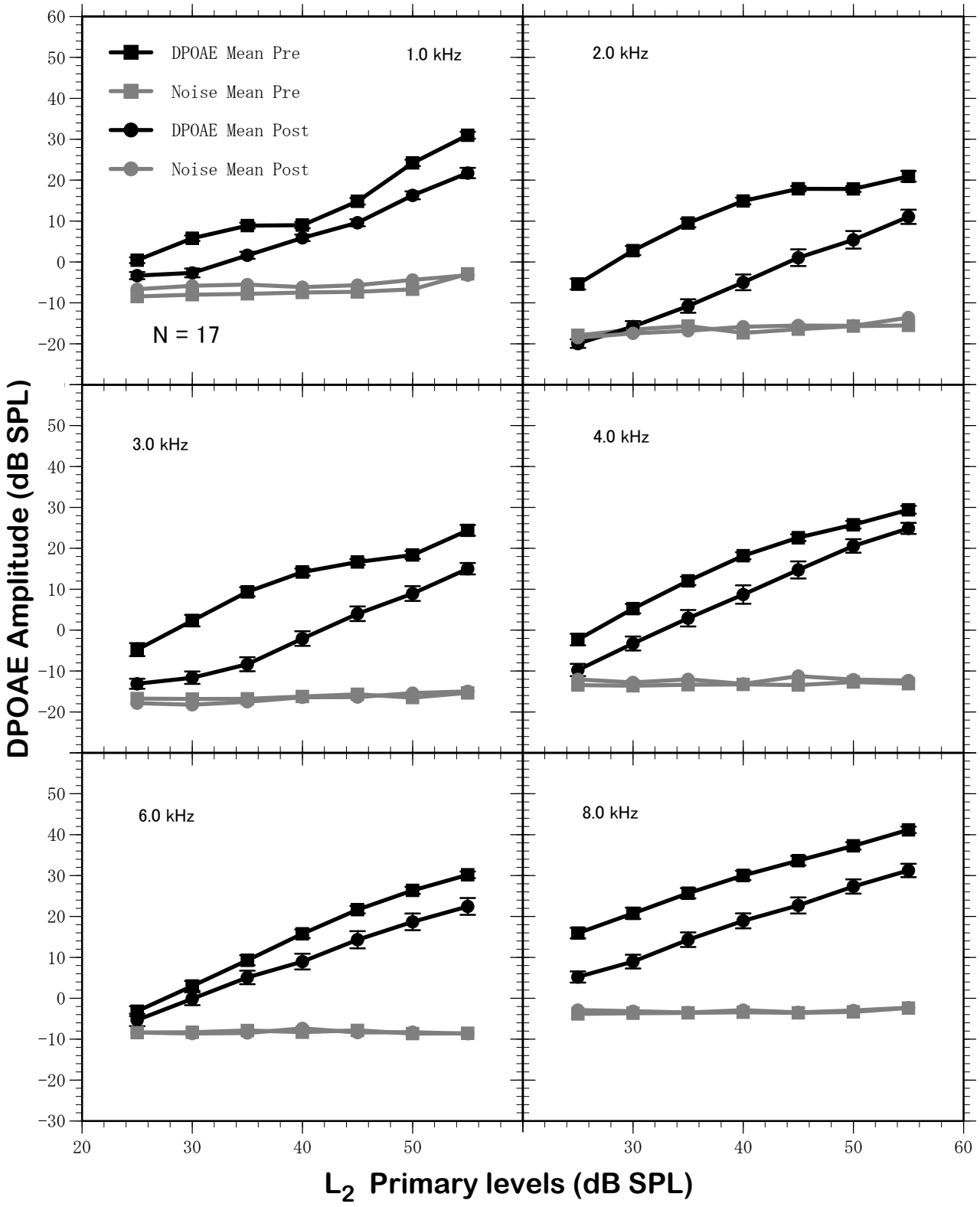


Figure 28. Phase II: Noise only combined control group mean (N=17) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2 for the combined noise only control group. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

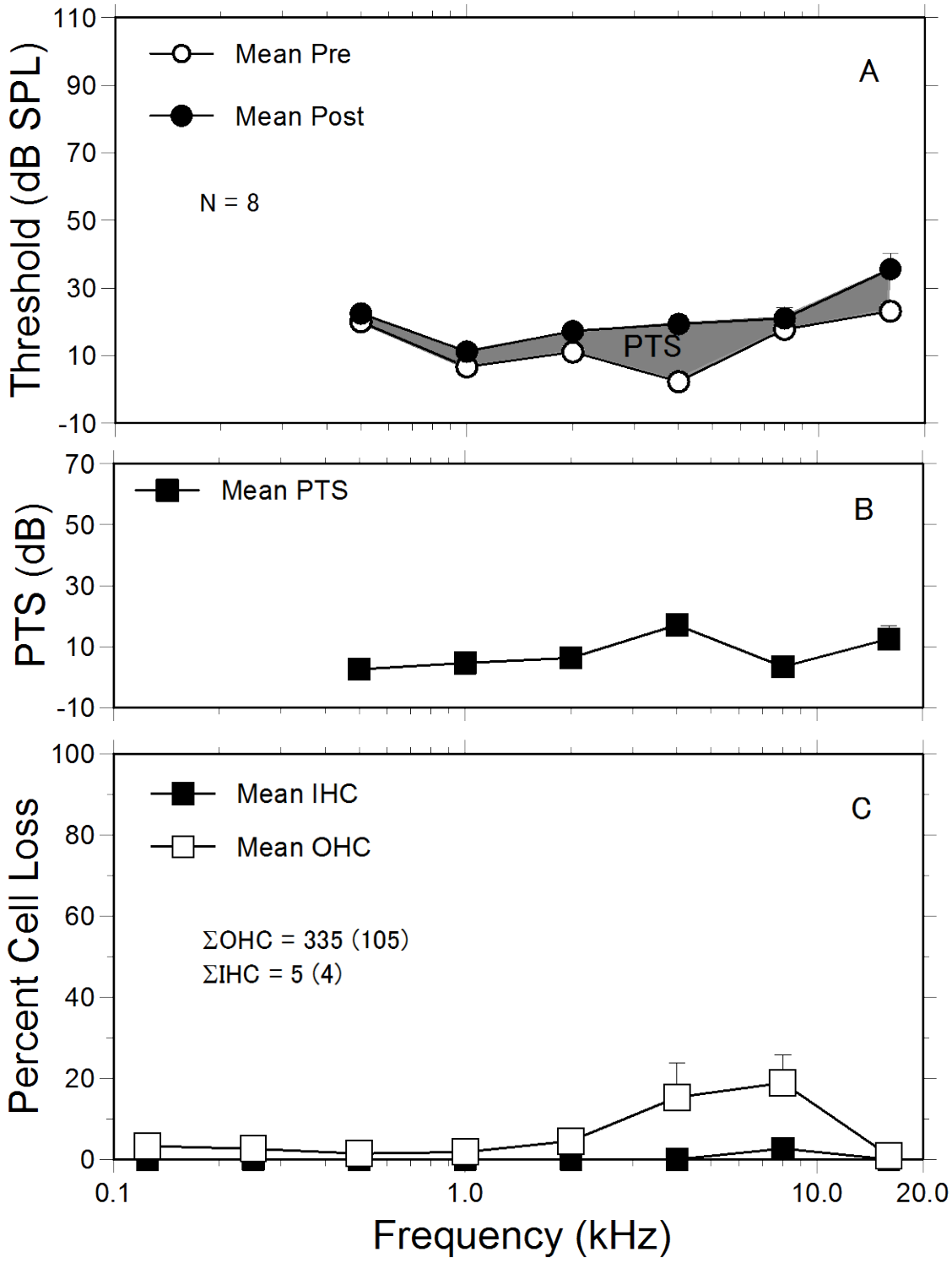


Figure 29. Phase II: Noise plus saline control group (N = 8) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

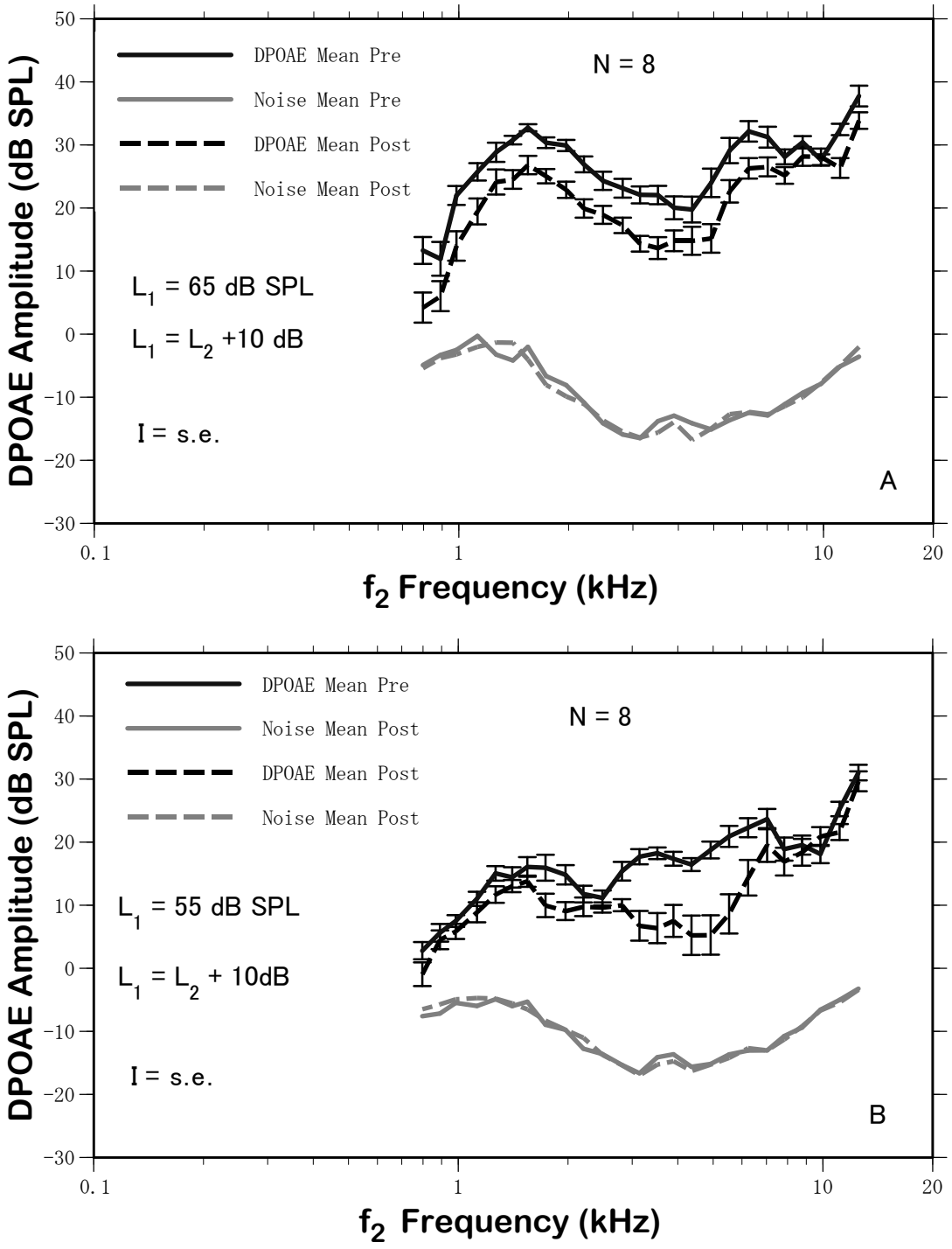


Figure 30. Phase II: Noise plus saline control group mean (N = 8) pre and post exposure cubic ($2f_1$- f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

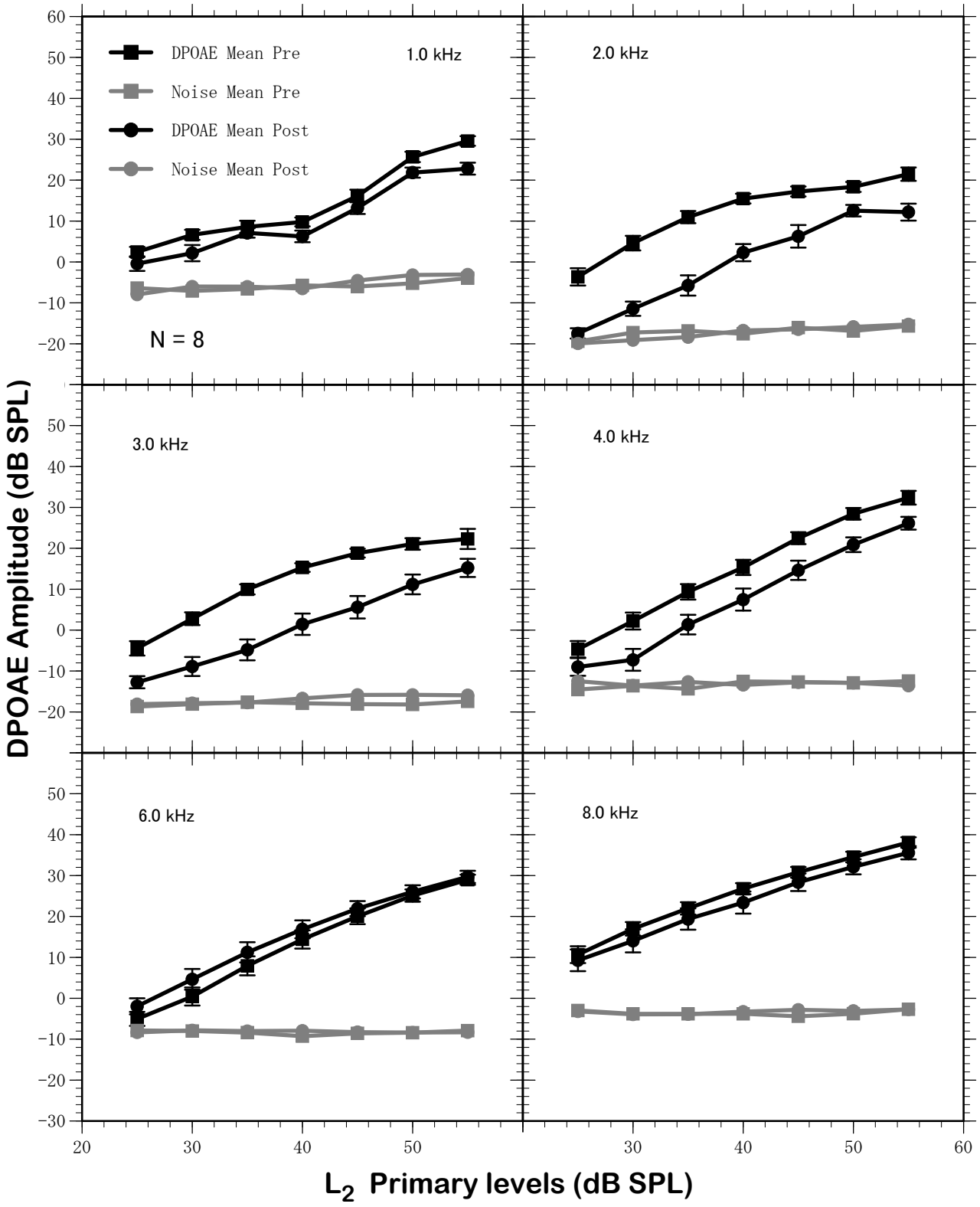


Figure 31. Phase II: Noise plus saline control group mean (N = 8) pre and post exposure cubic ($2f_1$- f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

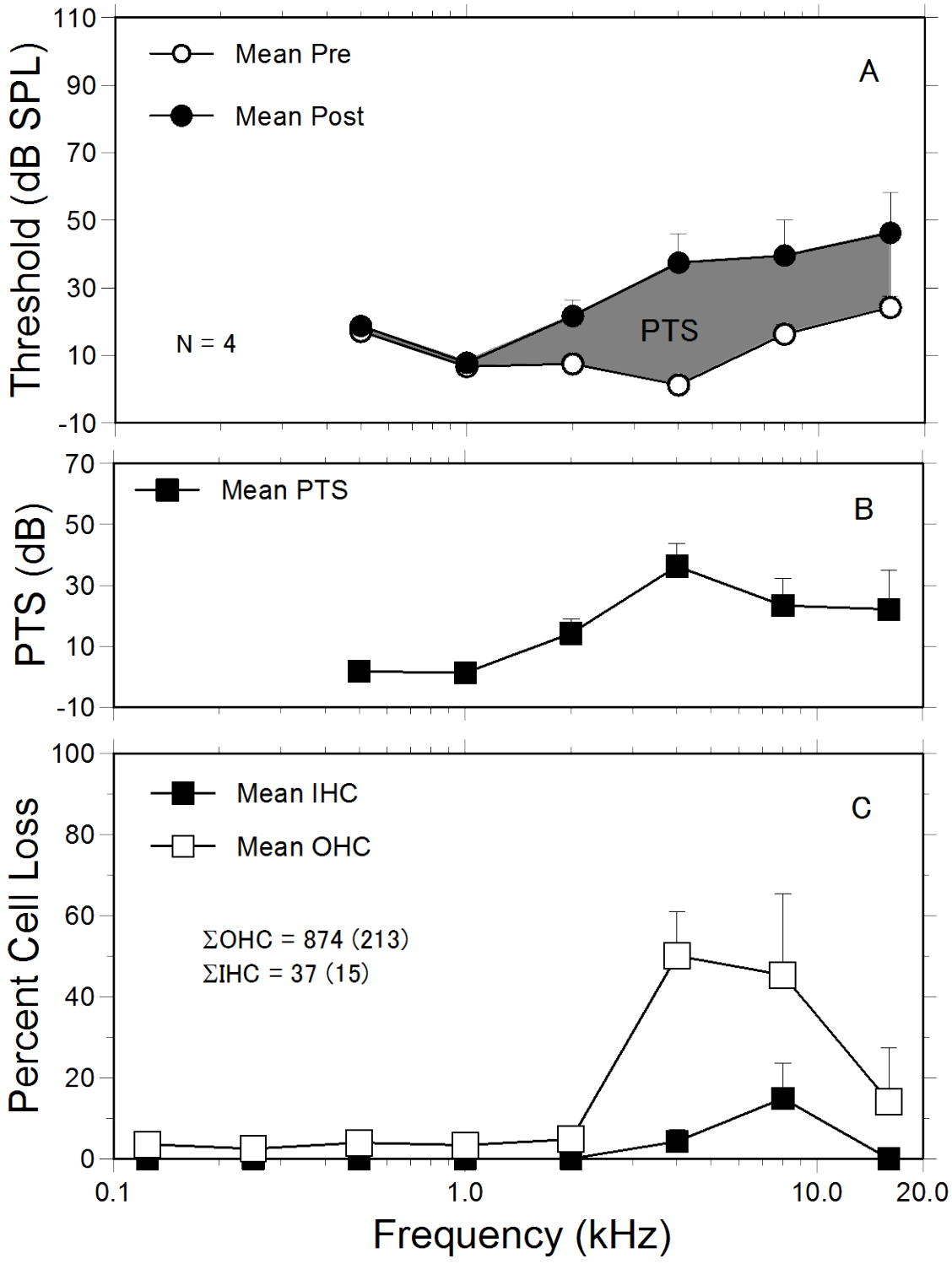


Figure 32. Phase II: Noise plus EDTA control group (N = 4) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer hair cell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

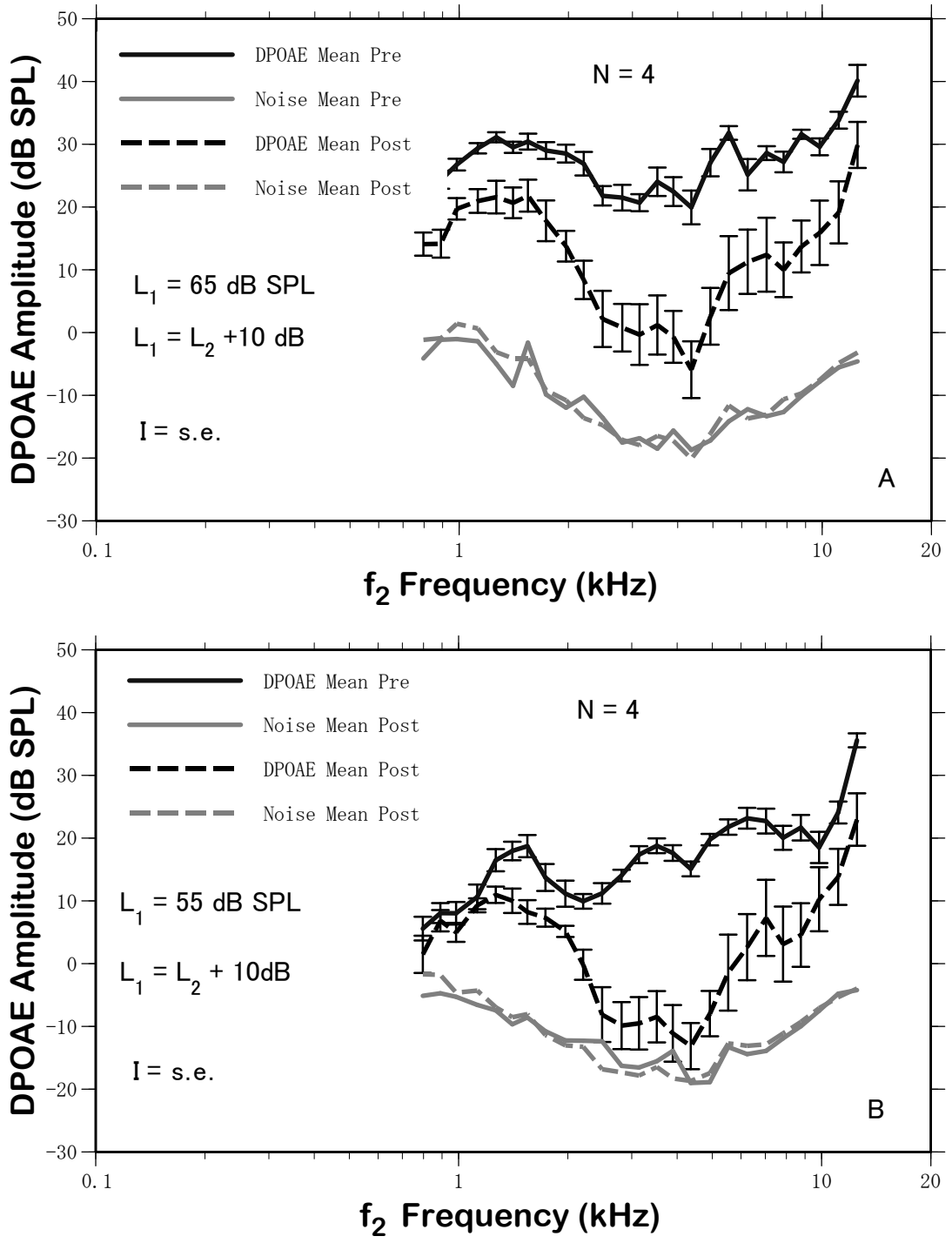


Figure 33. Phase II: Noise plus EDTA control group mean (N = 4) pre and post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

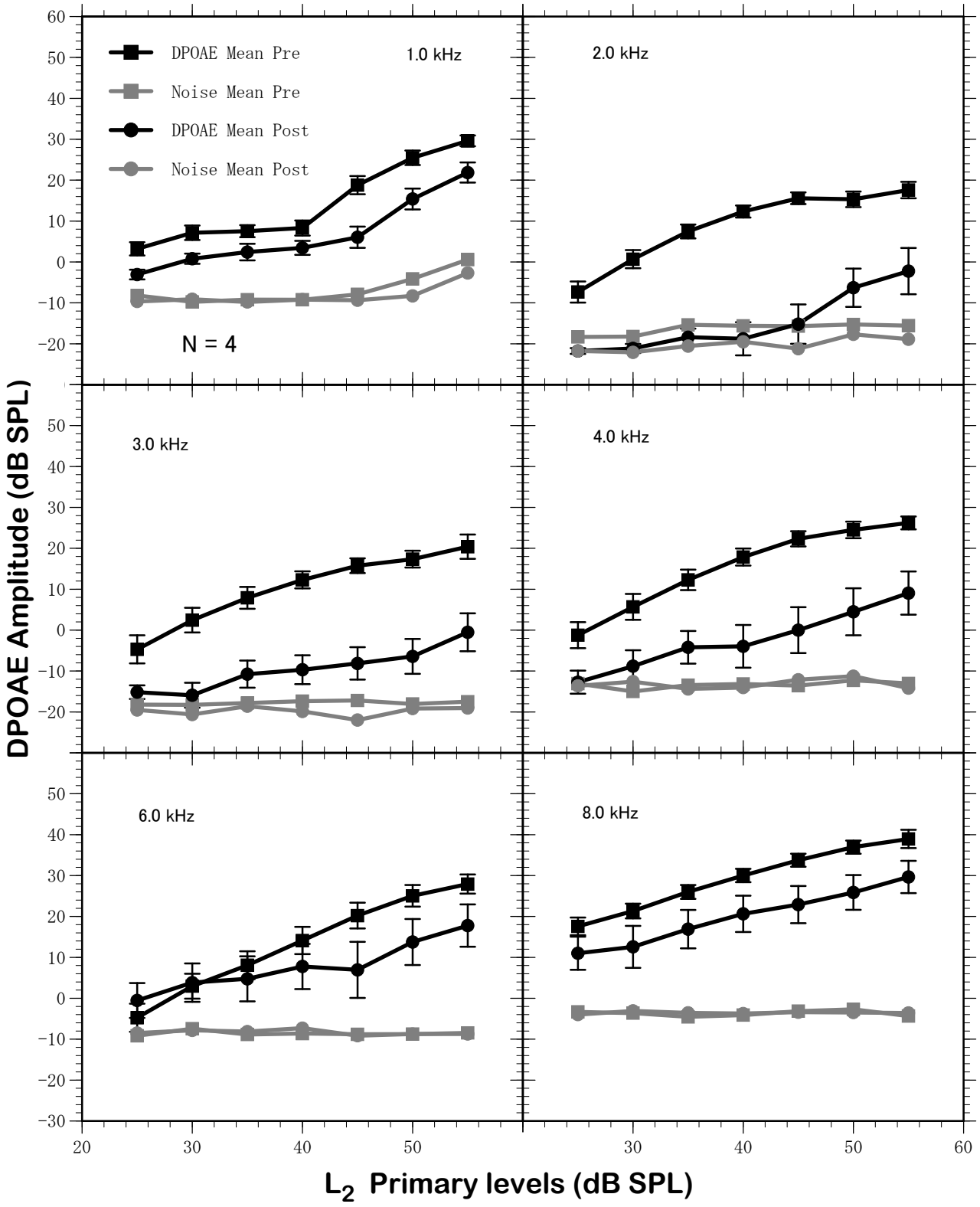


Figure 34. Phase II: Noise plus EDTA control group mean (N = 4) pre and post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 105 dB SPL for 6 hours. (I = s.e.)

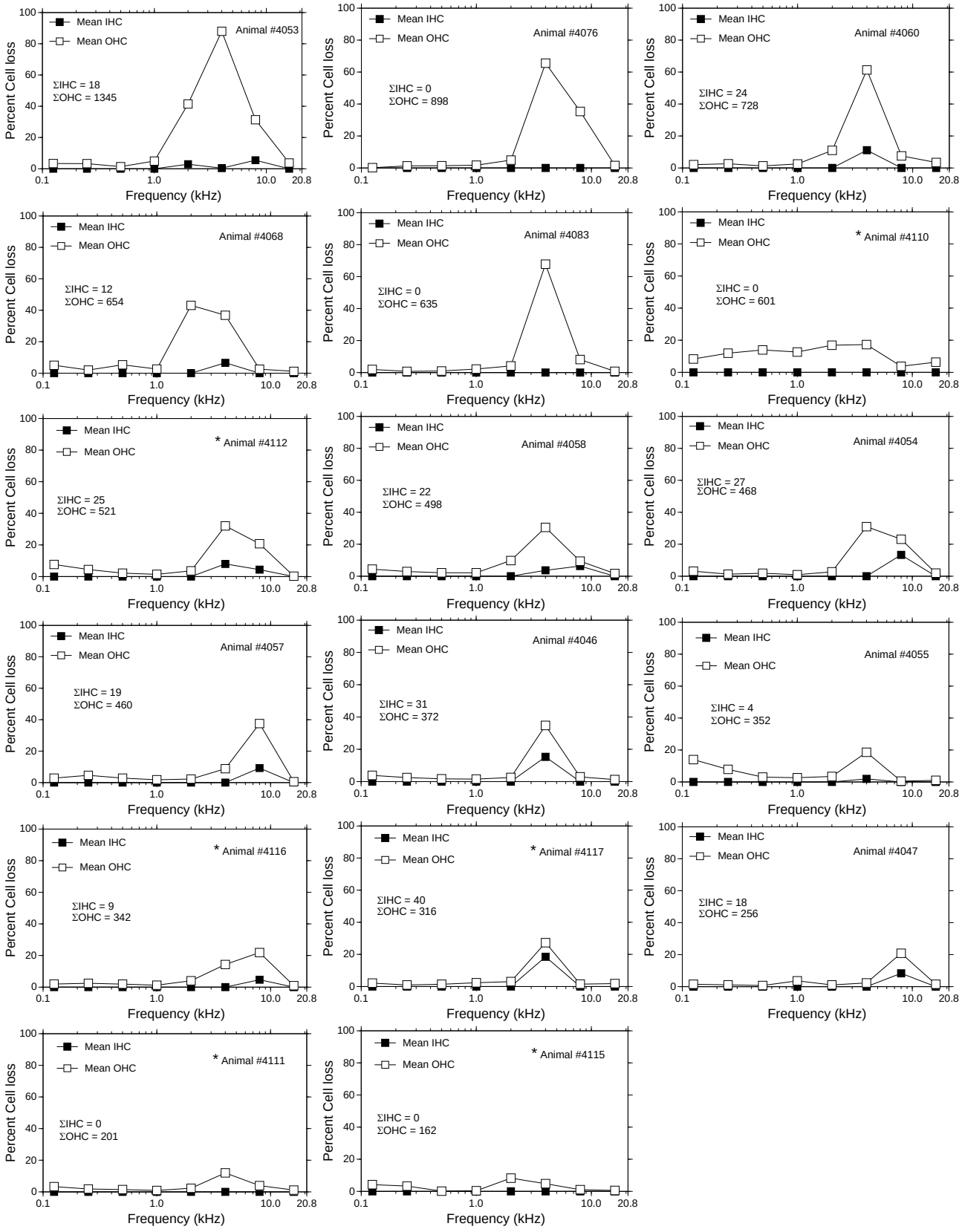


Figure 35. Phase II: Individual cochleagrams for the noise only control group (N=17) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. The cochleagrams are arranged in a descending order of sensory cell loss. ΣOHC and ΣIHC present the total number of outer and inner hair cells lost respectively. * refers to animals exposed in the reconfigured speaker/cage set up.

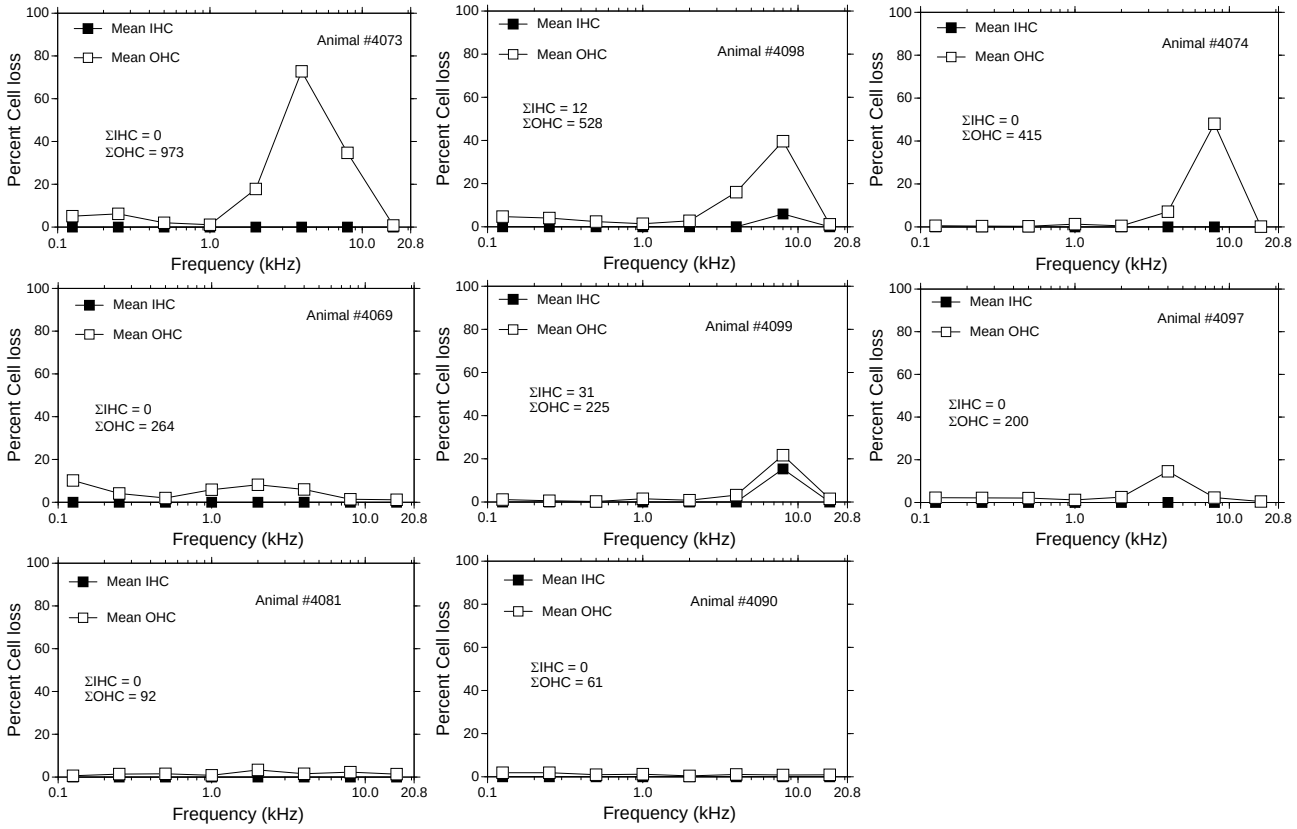


Figure 36. Phase II: Individual cochleagrams for the noise plus saline control group (N=8) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. The cochleagrams are arranged in a descending order of sensory cell loss. ΣOHC and ΣIHC present the total number of outer and inner hair cells lost respectively.

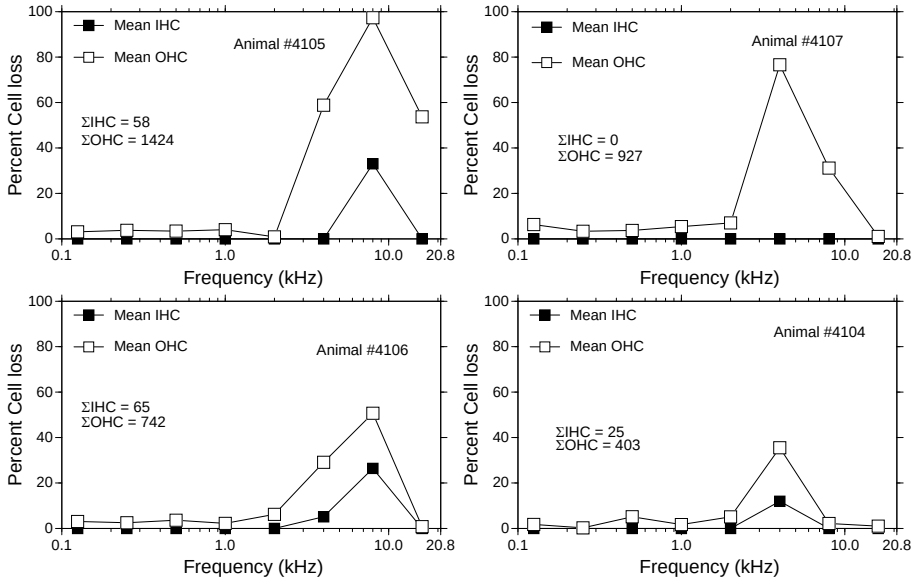


Figure 37. Phase II: Individual cochleagrams for the noise plus EDTA control group (N=4) exposed to a 105 dB SPL, 4 kHz octave band of noise for 6 hours. The cochleagrams are arranged in a descending order of sensory cell loss. ΣOHC and ΣIHC present the total number of outer and inner hair cells lost respectively.

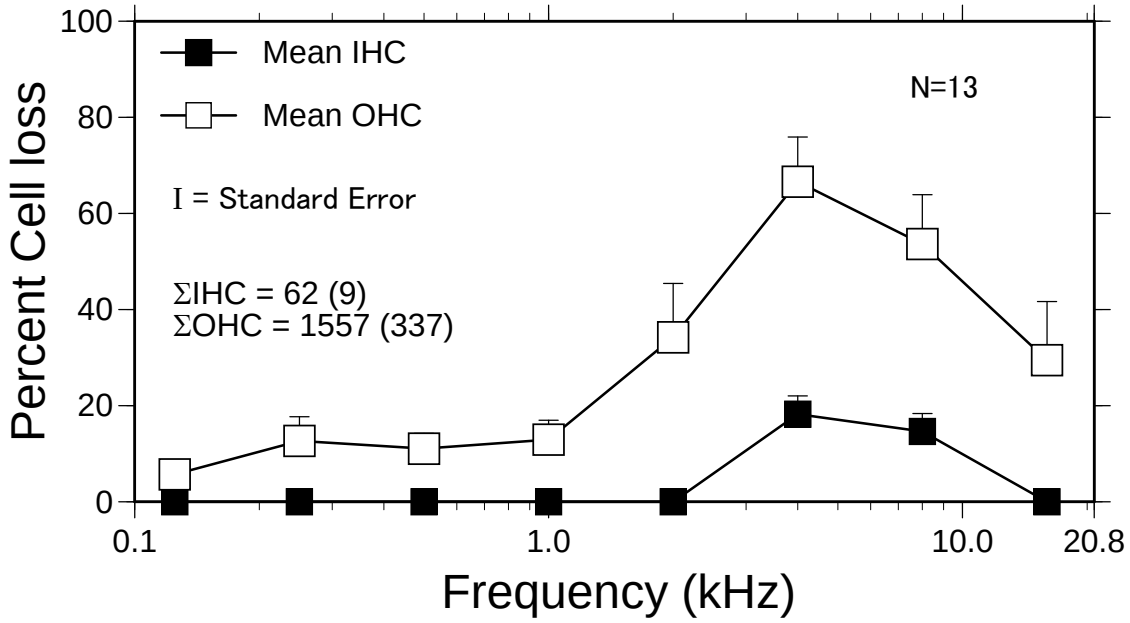


Figure 38. The group mean percent sensory cell loss for N = 13 subjects exposed to the 108 dB SPL, 4 kHz OBN noise for 6 hours. The animals were euthanized 10 days after the exposure. (T = standard error).

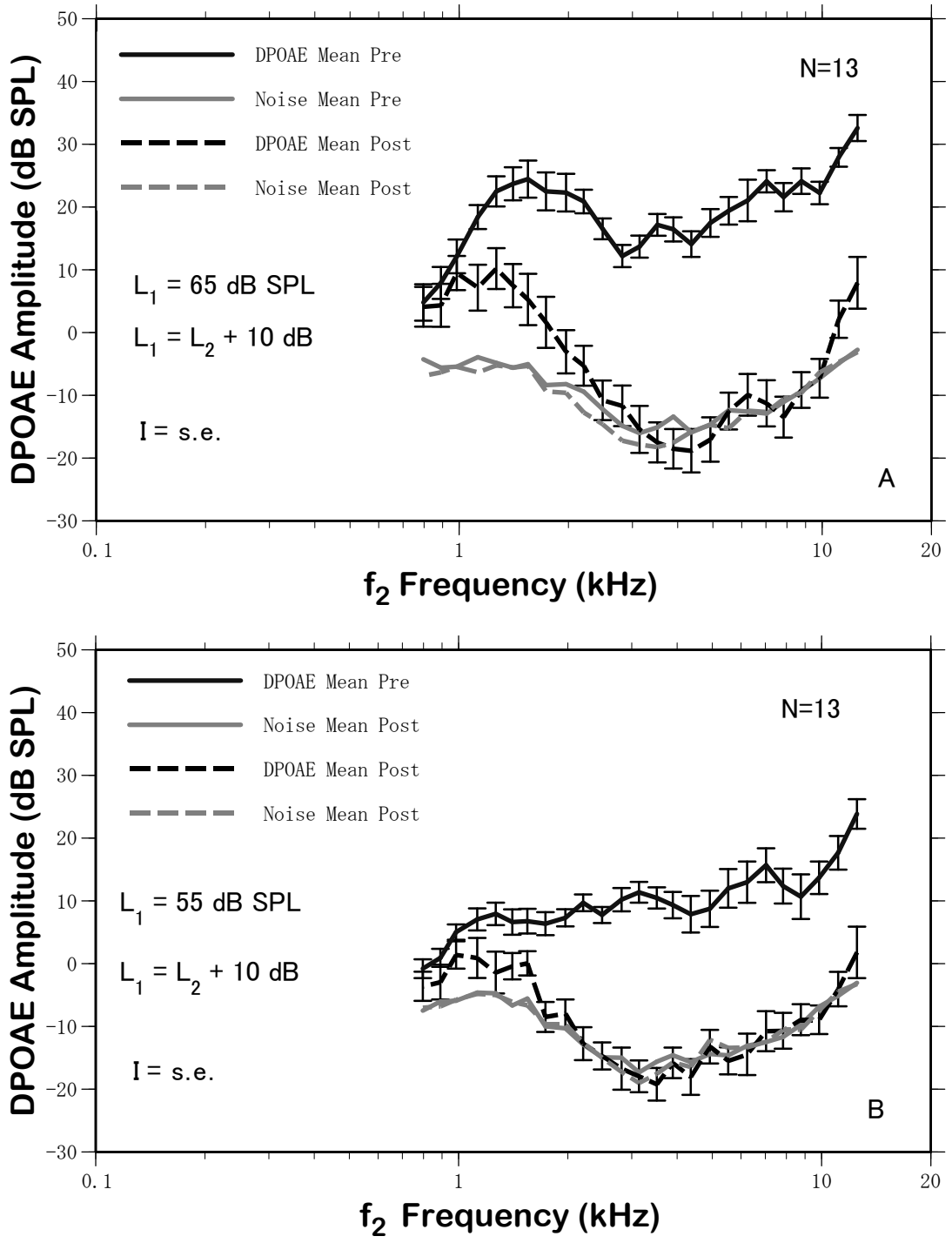


Figure 39. Group mean (N = 13) pre and 10-day post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2 + 10$ dB and $f_2/f_1 = 1.22$. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

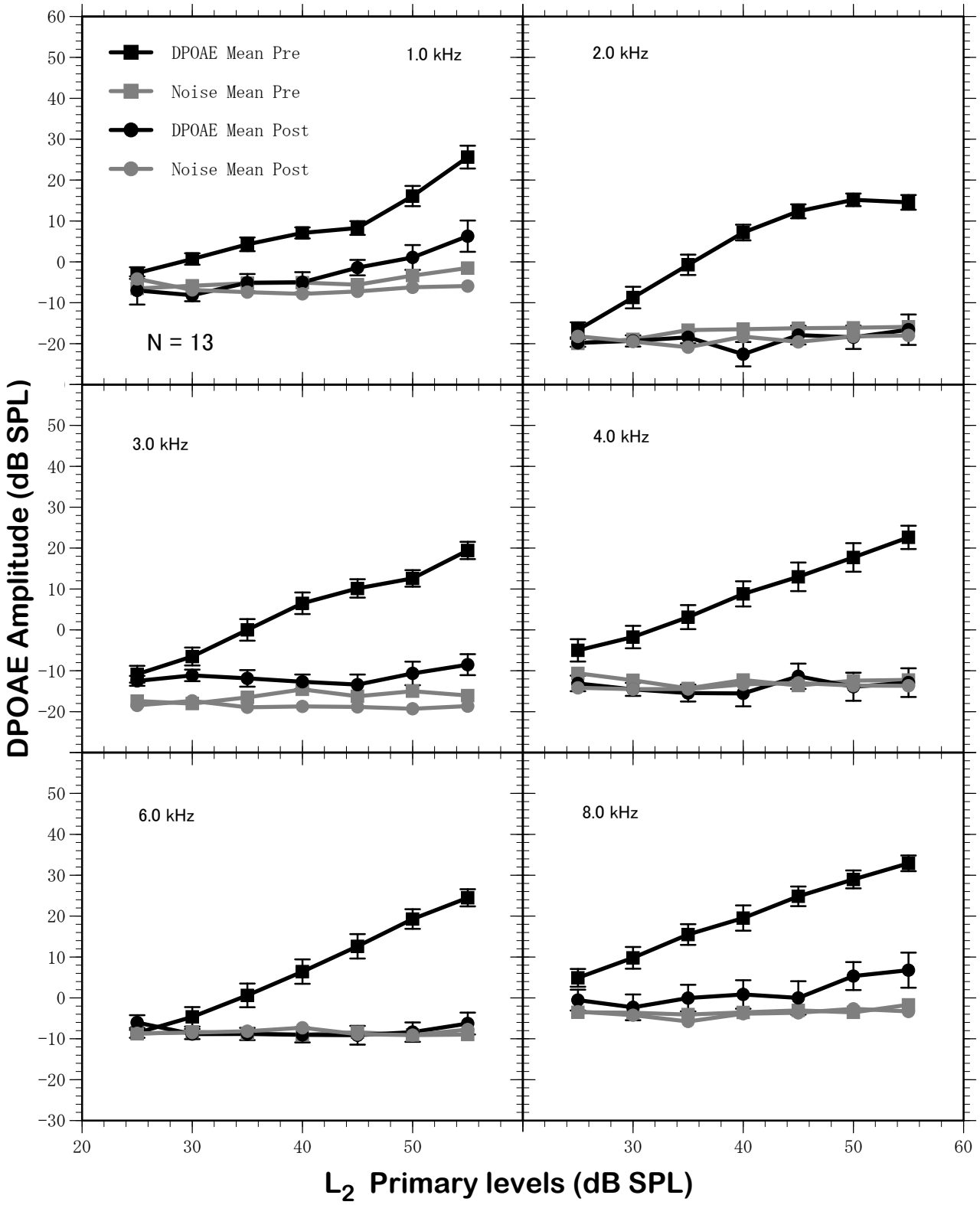


Figure 40. Group mean (N = 13) pre and 10-day post exposure cubic ($2f_1 - f_2$) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

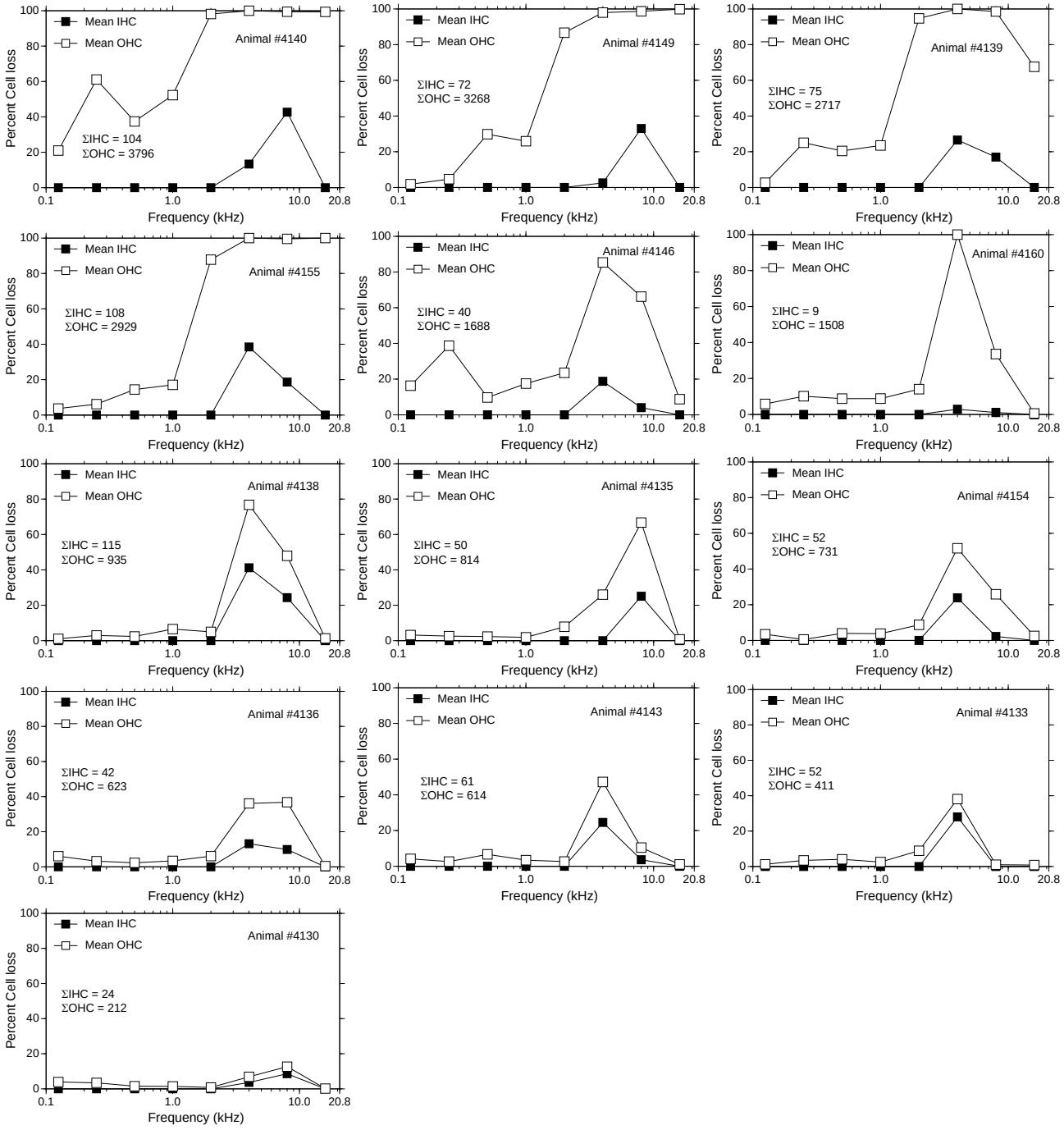


Figure 41. Individual cochleograms for the 13 chinchillas exposed to the 108 dB, 4 kHz OBN. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 10 days post exposure.

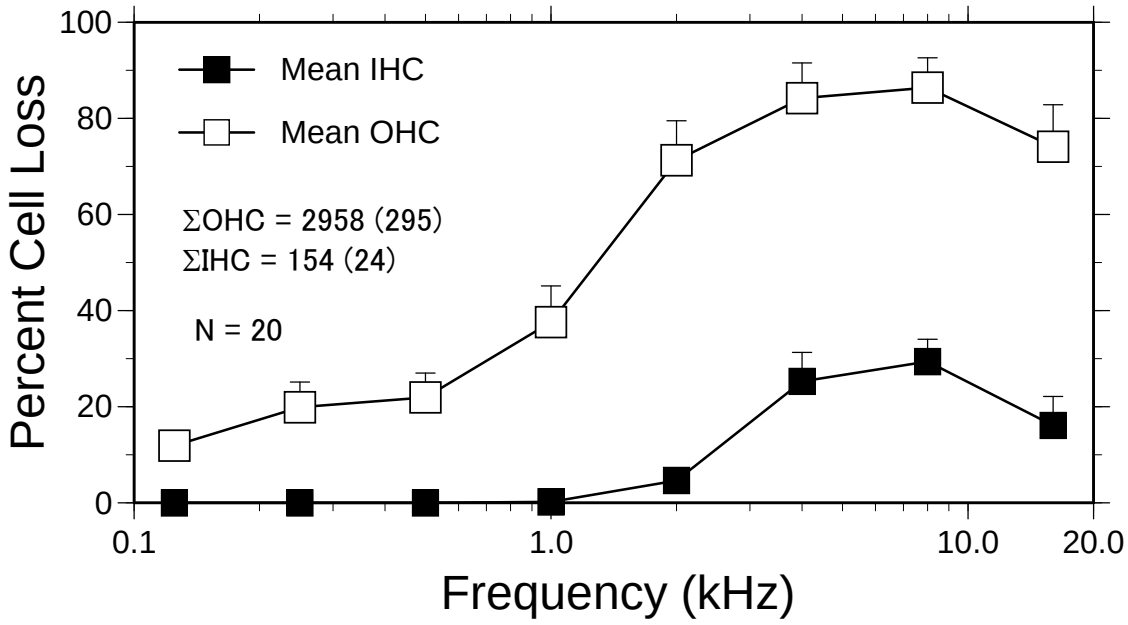


Figure 42. The group (N=20) mean percent inner and outer haircell (IHC, OHC) loss for animals exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error). (Lesion calibration animals; no audiometric or emission data collected.)

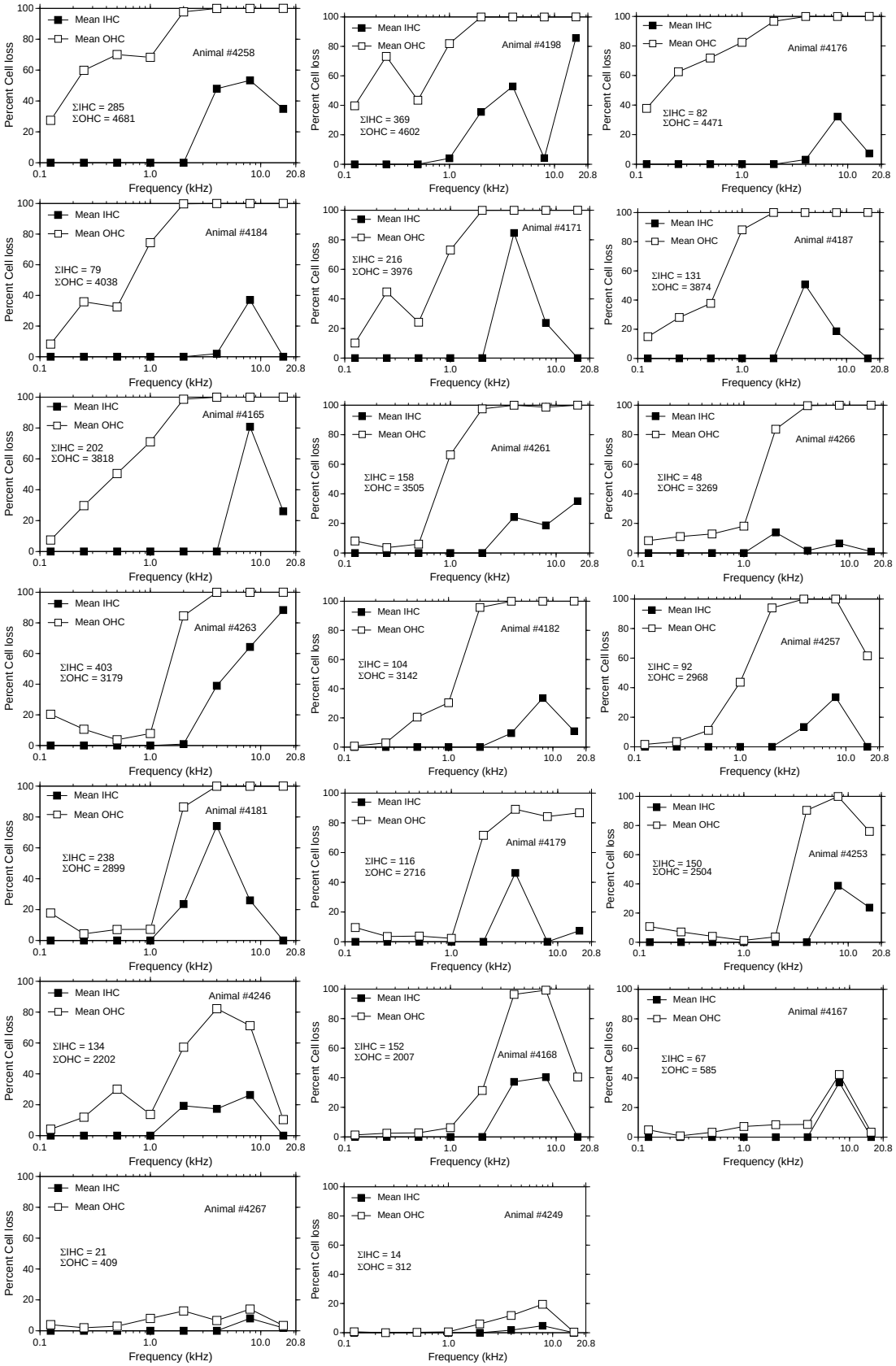


Figure 43. Individual cochleograms for the 20 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells. (Lesion calibration animals; no audiometric data collected.)

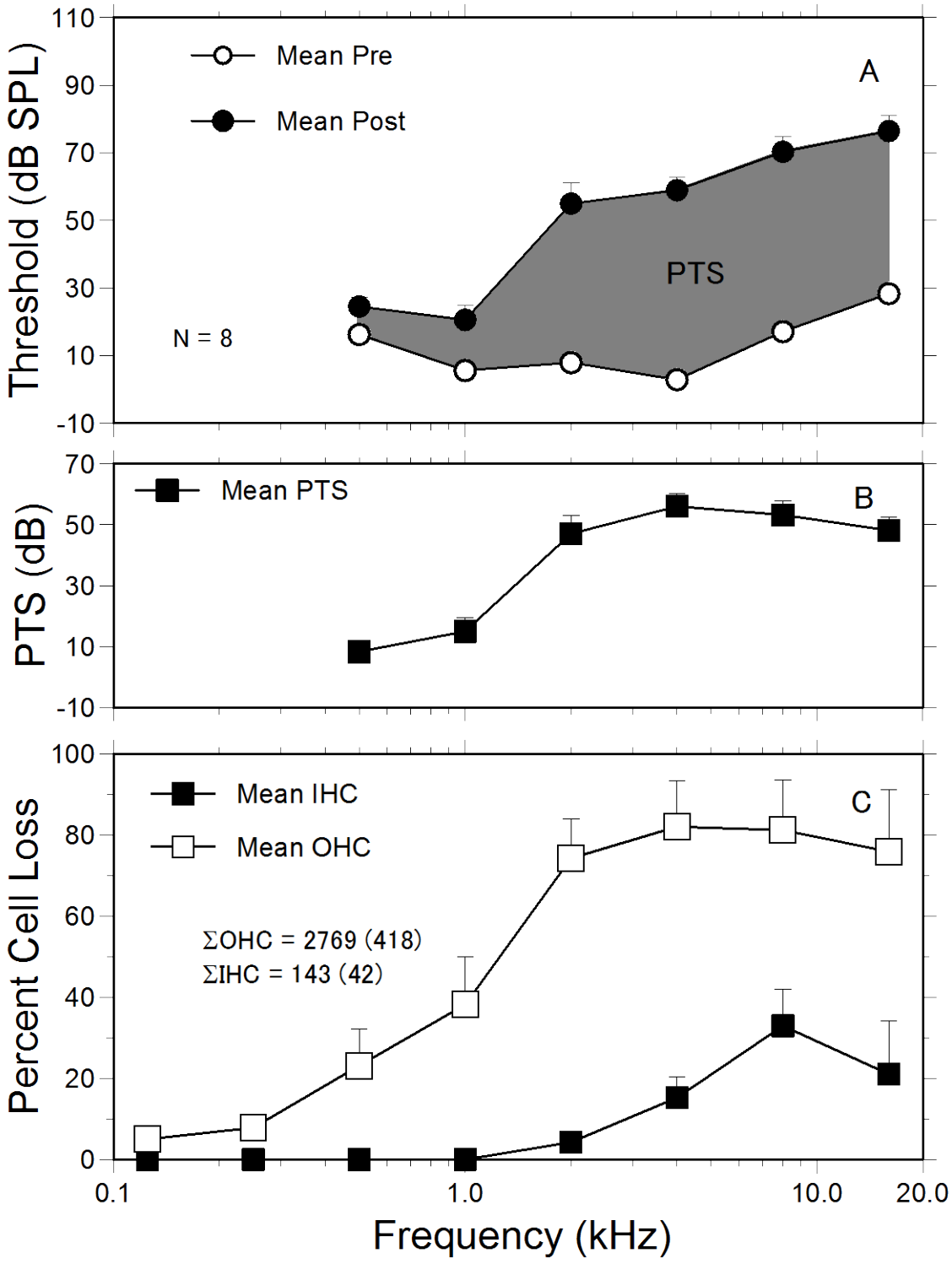


Figure 44. Phase II: Noise only control group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

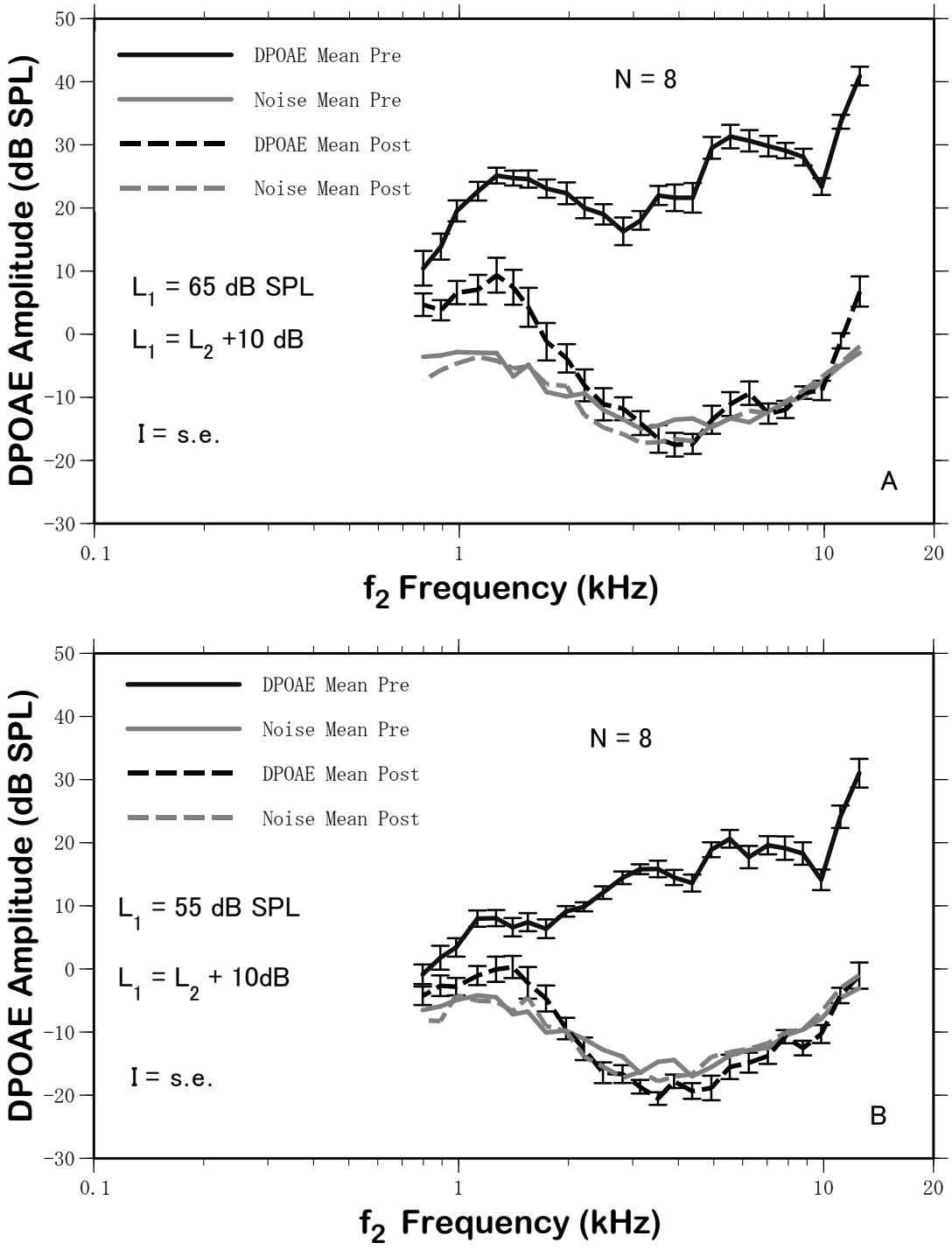


Figure 45. Phase II: Noise only control group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

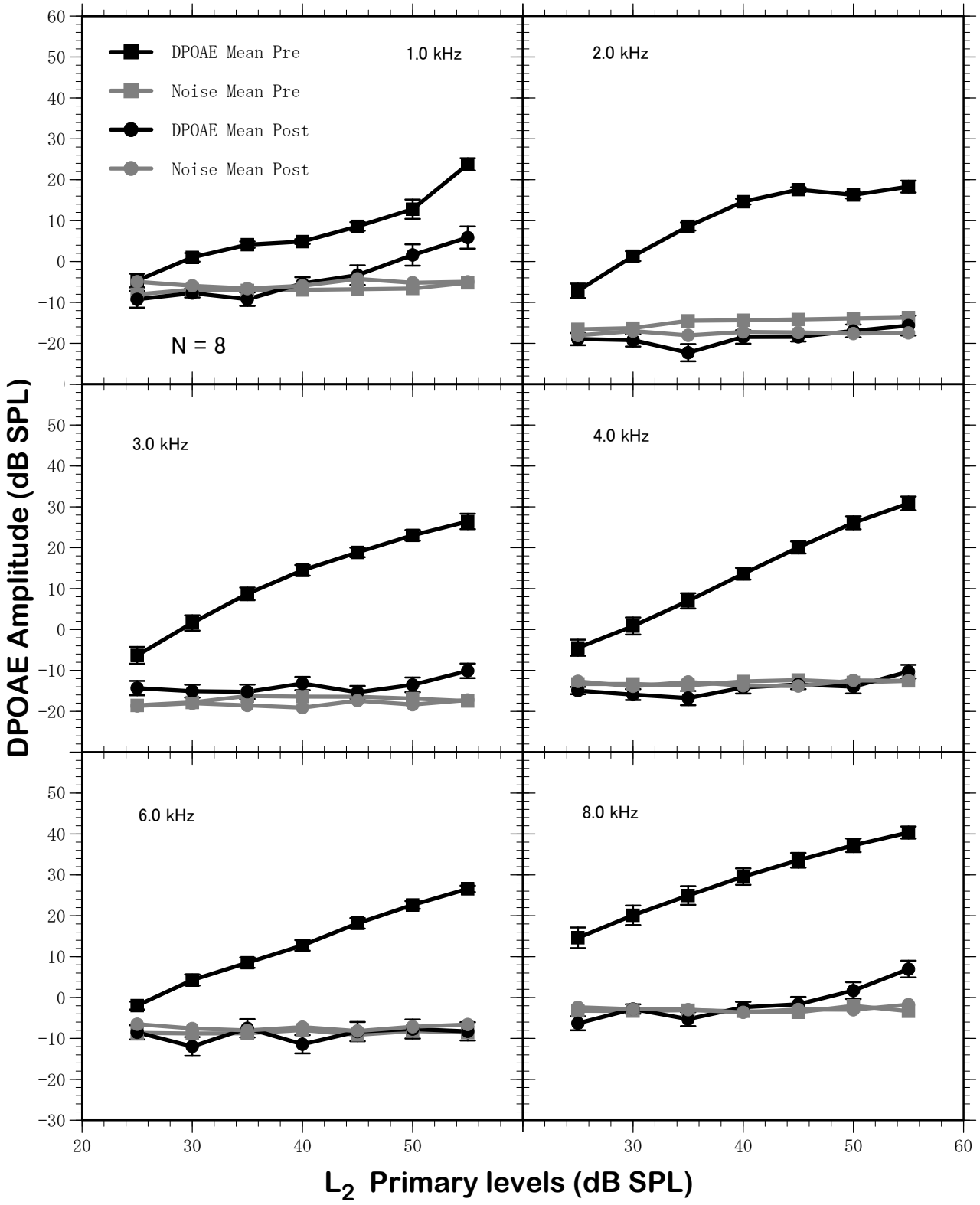


Figure 46. Phase II: Noise only control group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

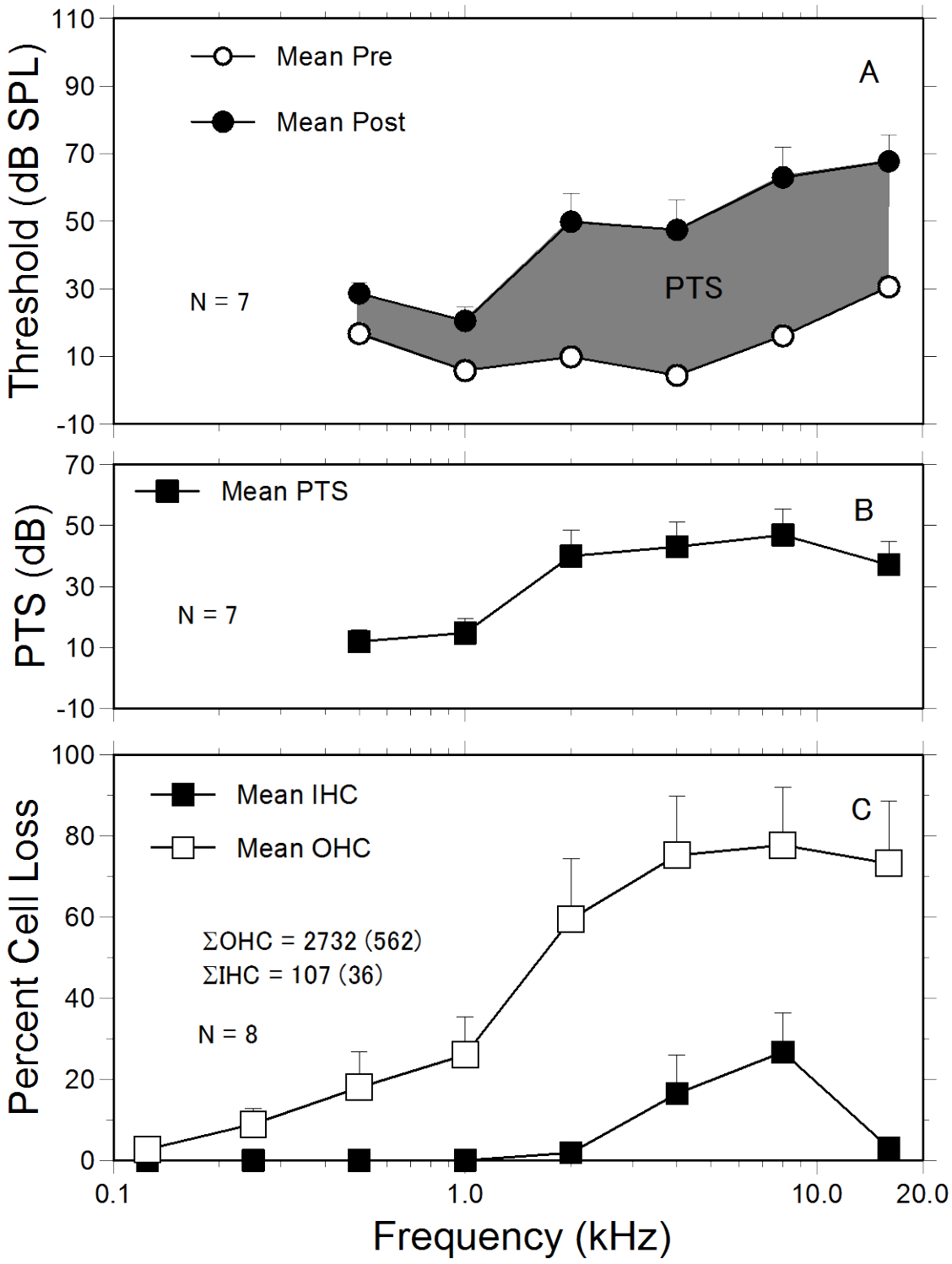


Figure 47. Phase II: Noise plus saline control group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

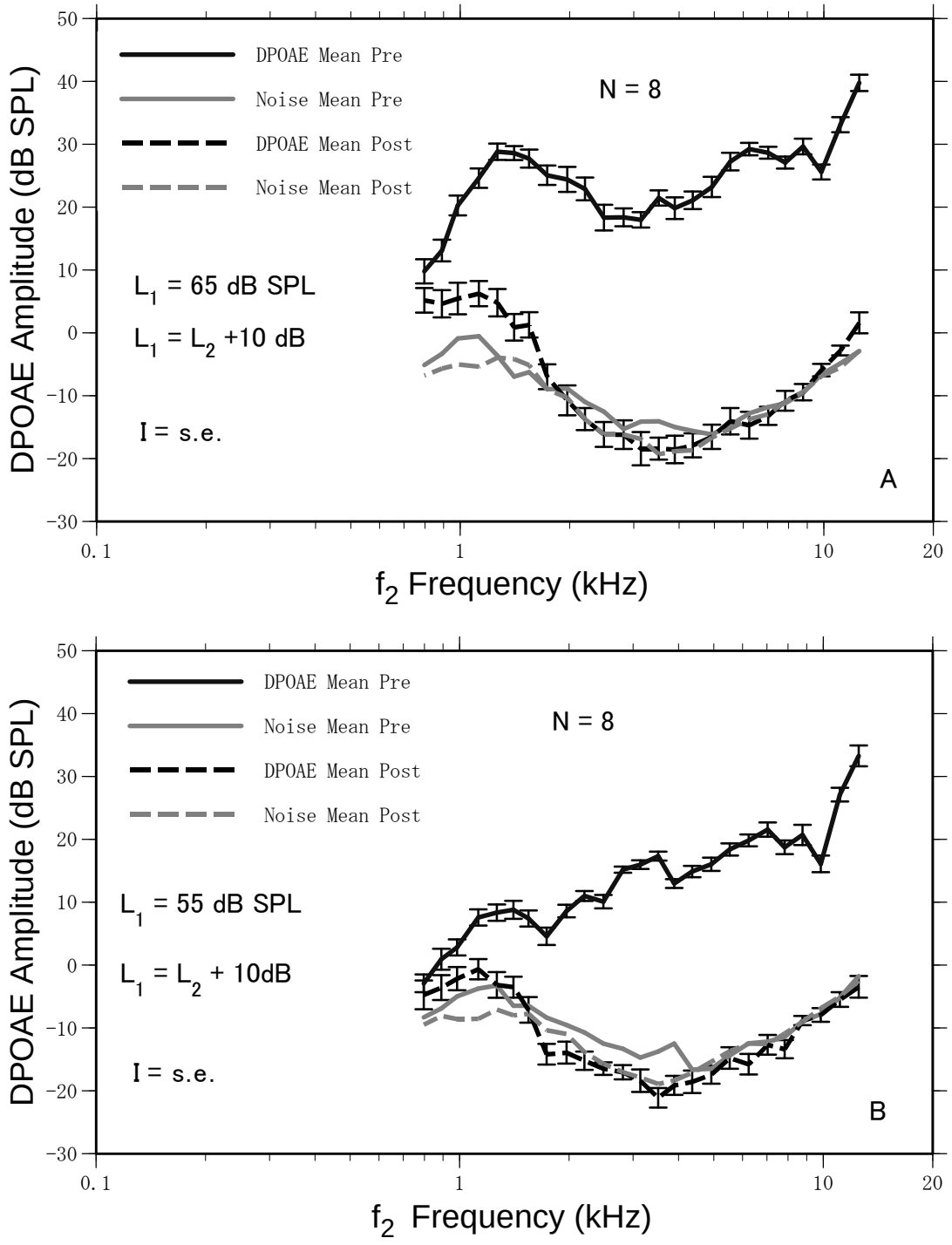


Figure 48. Phase II: Noise plus saline control group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

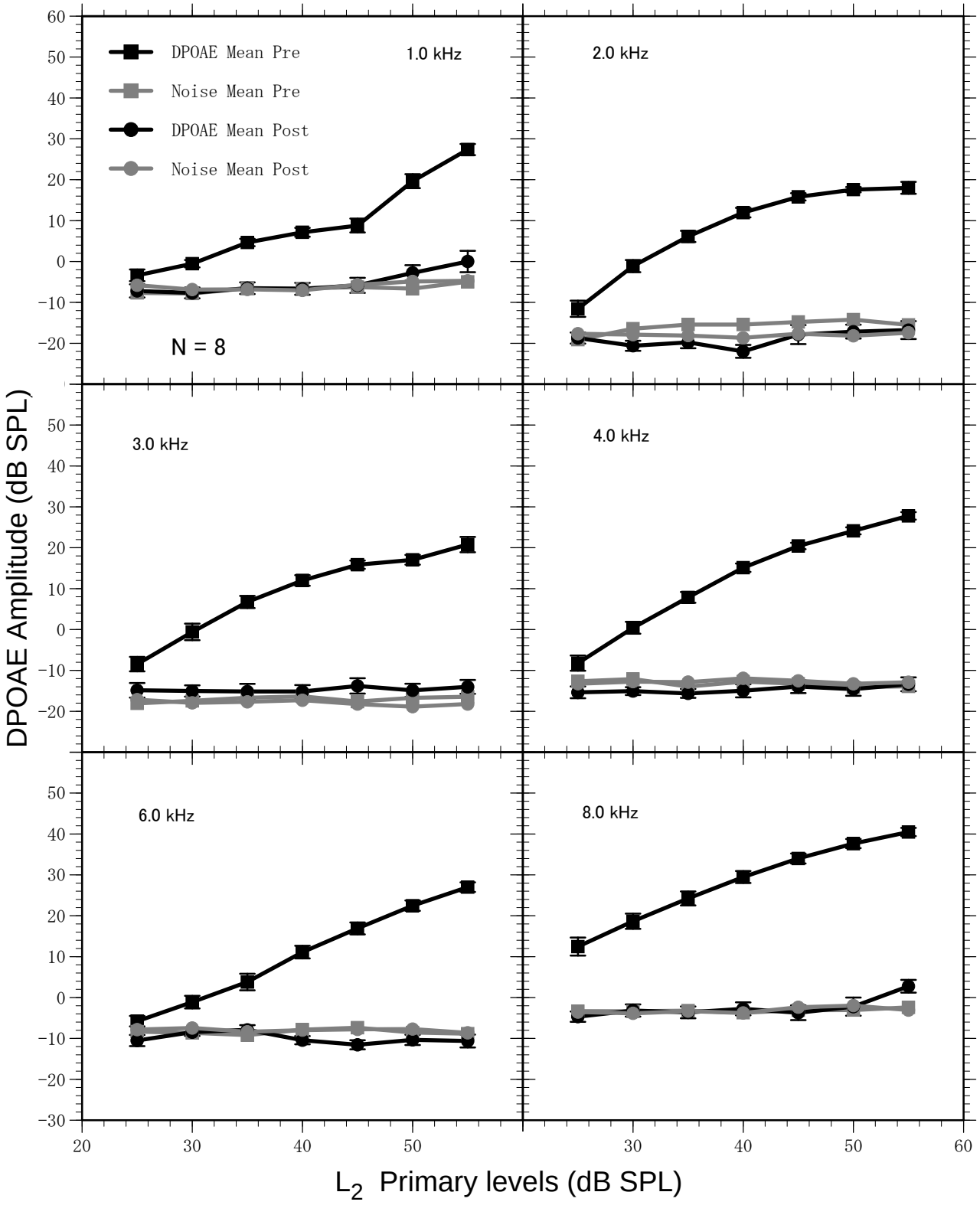


Figure 49. Phase II: Noise plus saline control group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

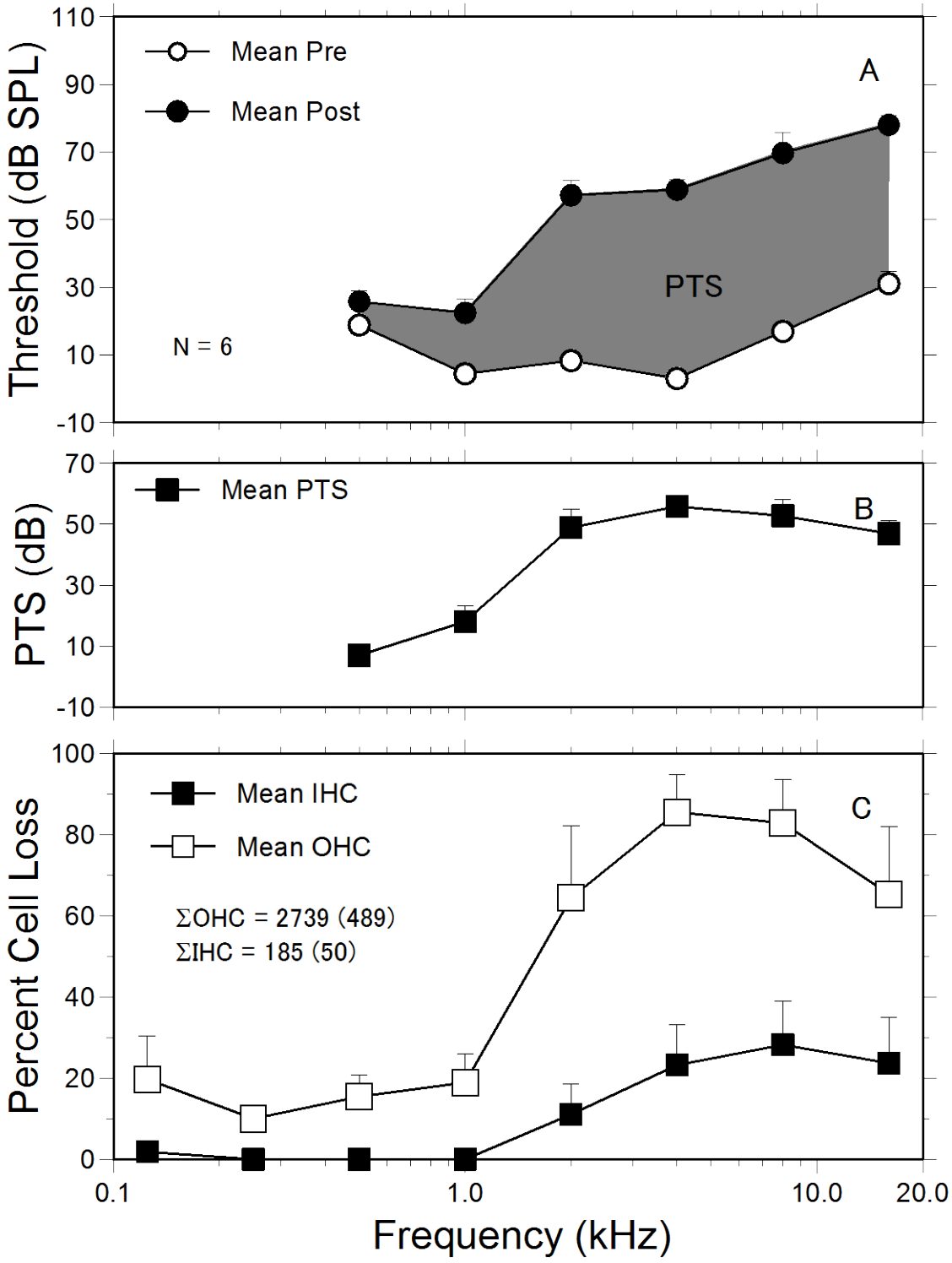


Figure 50. Phase II: Noise plus EDTA control group (N=6) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

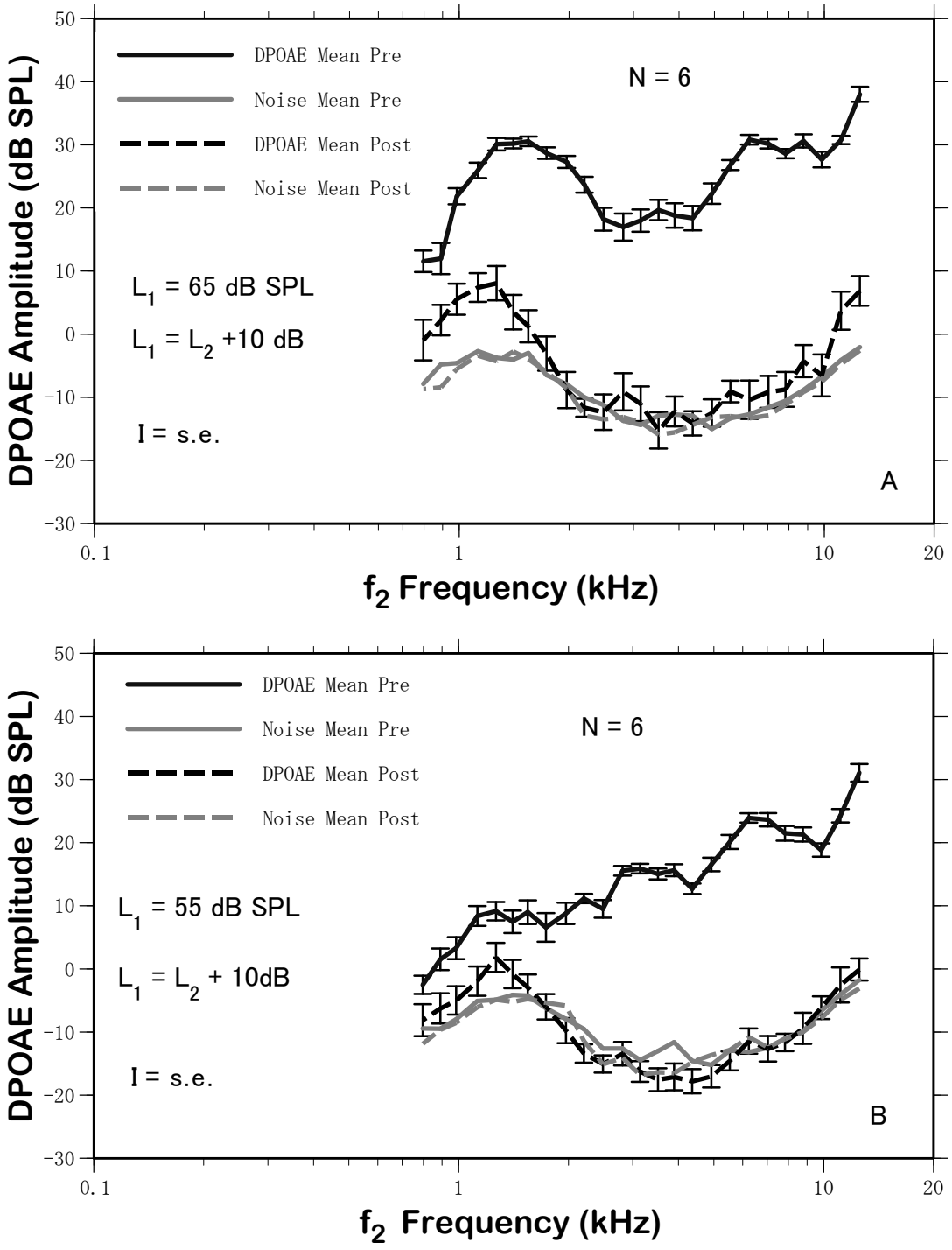


Figure 51. Phase II: Noise plus EDTA control group mean (N=6) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

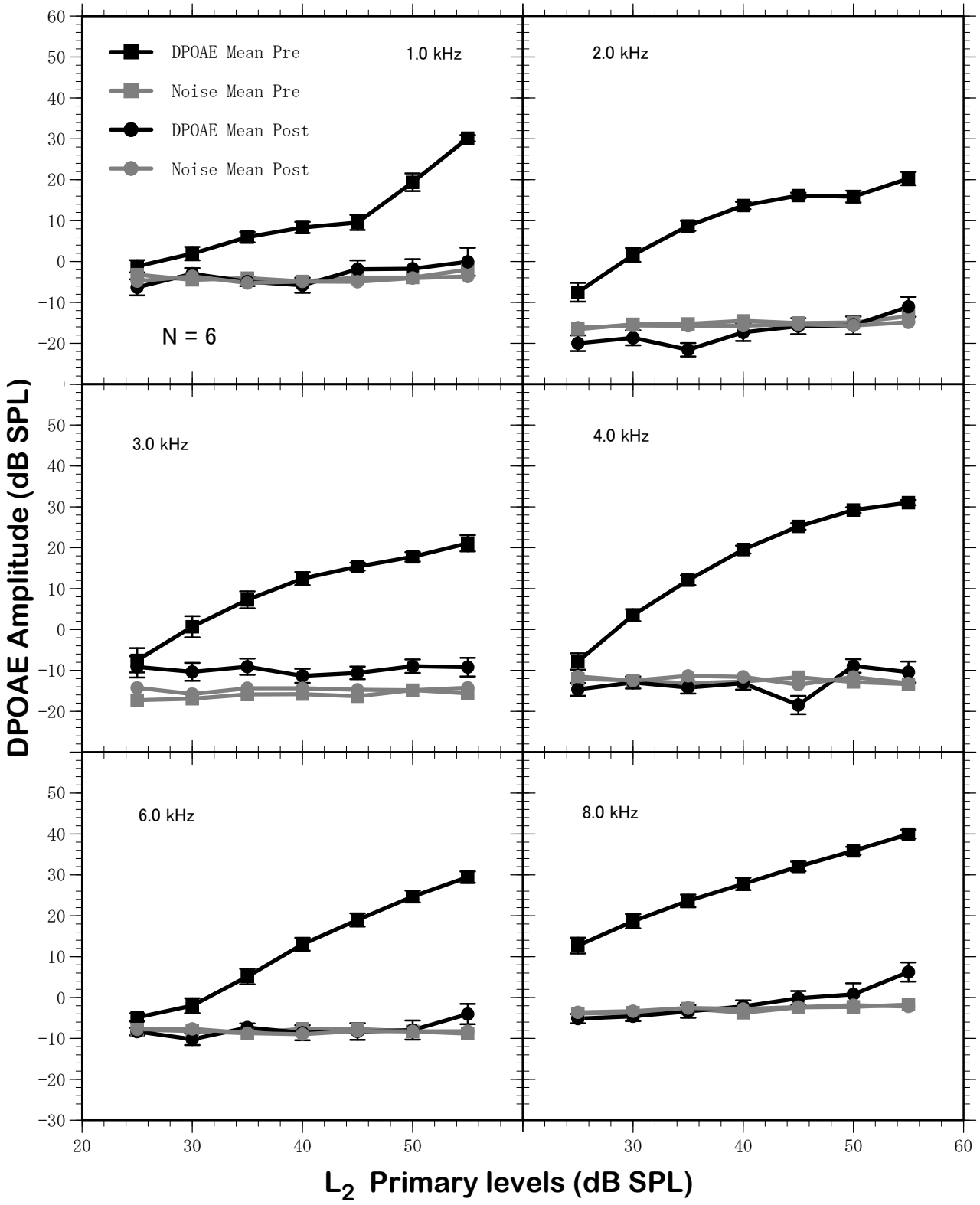


Figure 52. Phase II: Noise plus EDTA control group mean (N=6) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

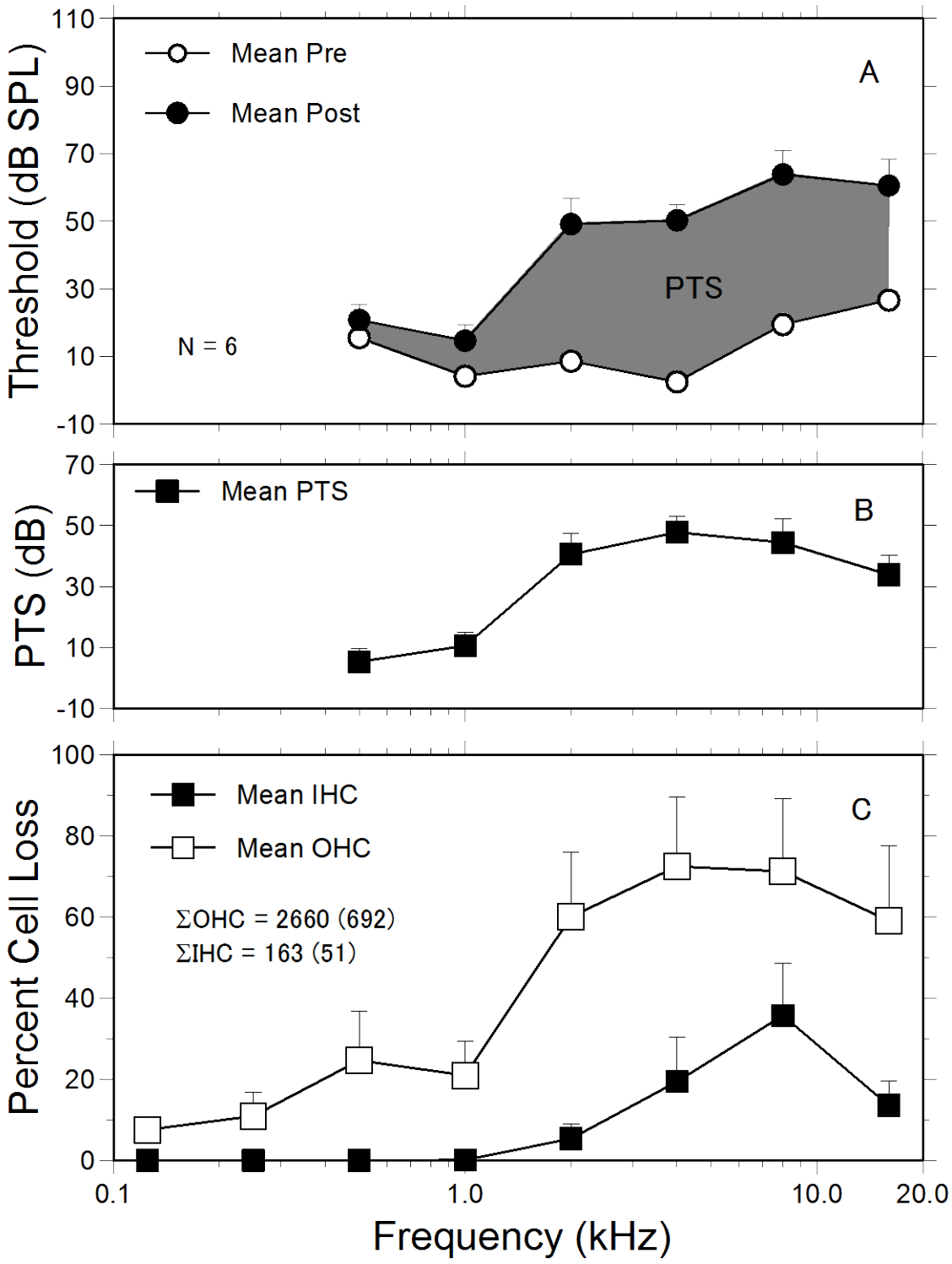


Figure 53. Phase II: Noise plus saline plus DMSO control group (N=6) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

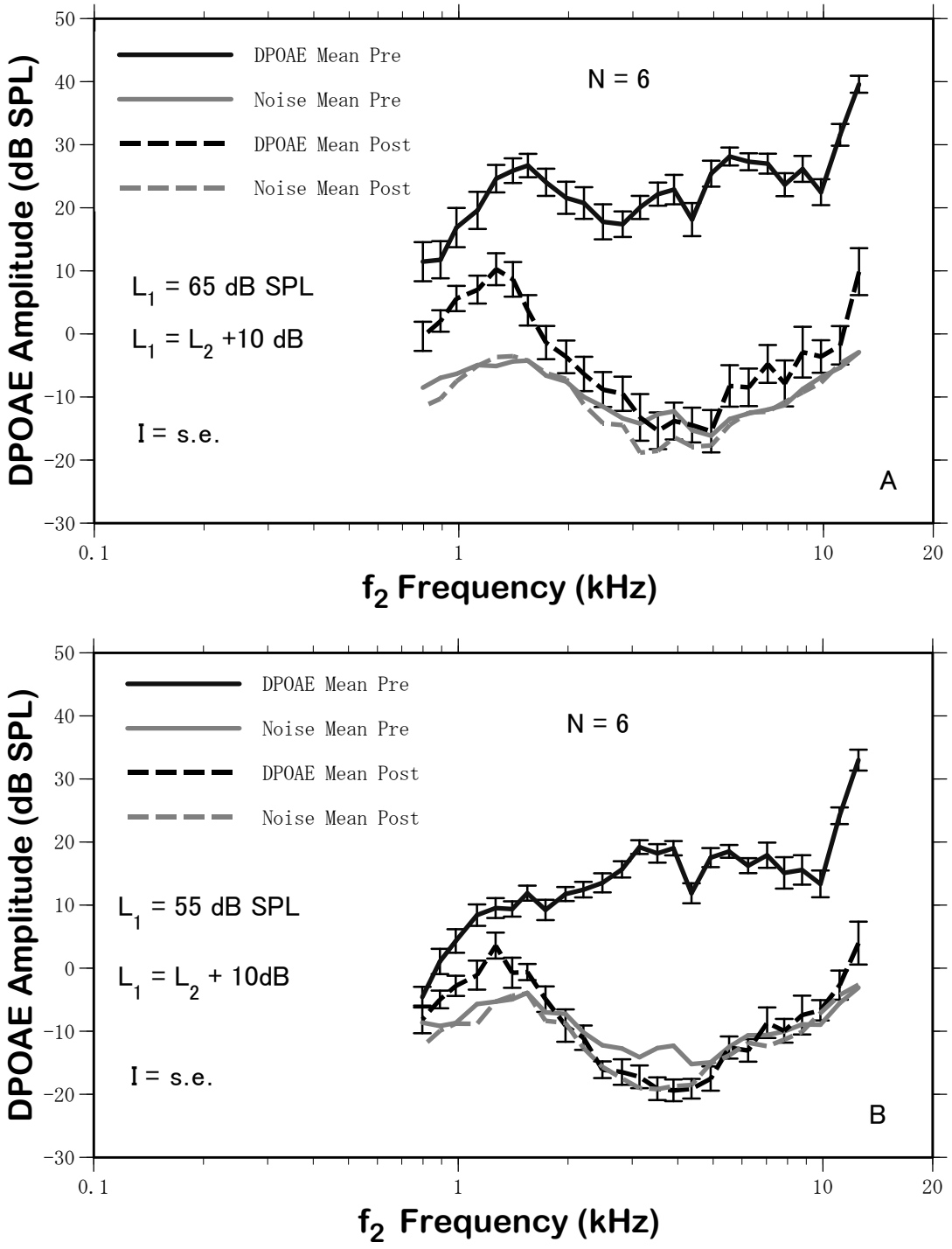


Figure 54. Phase II: Noise plus saline plus DMSO control group mean (N=6) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

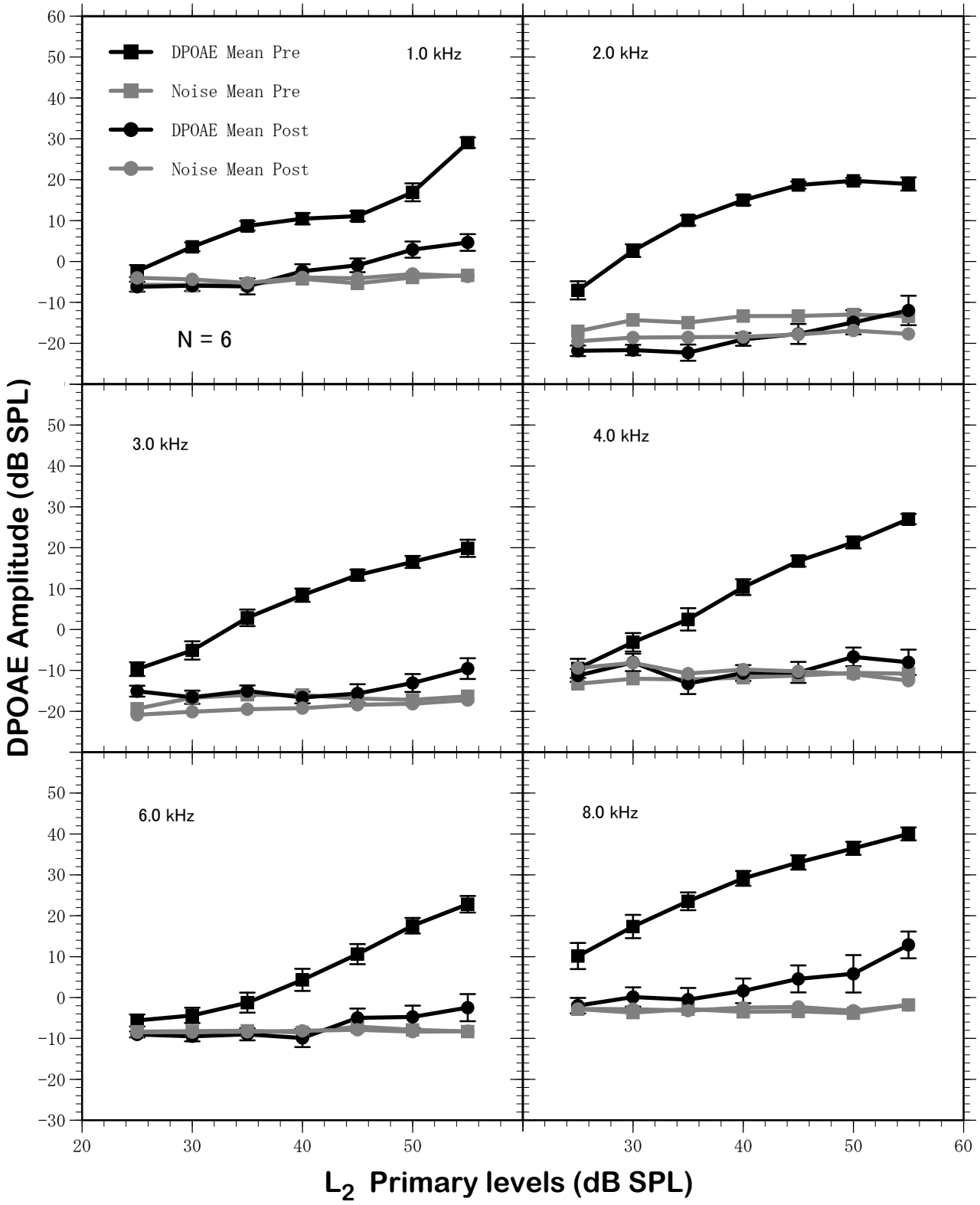


Figure 55. Phase II: Noise plus saline plus DMSO control group mean (N=6) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

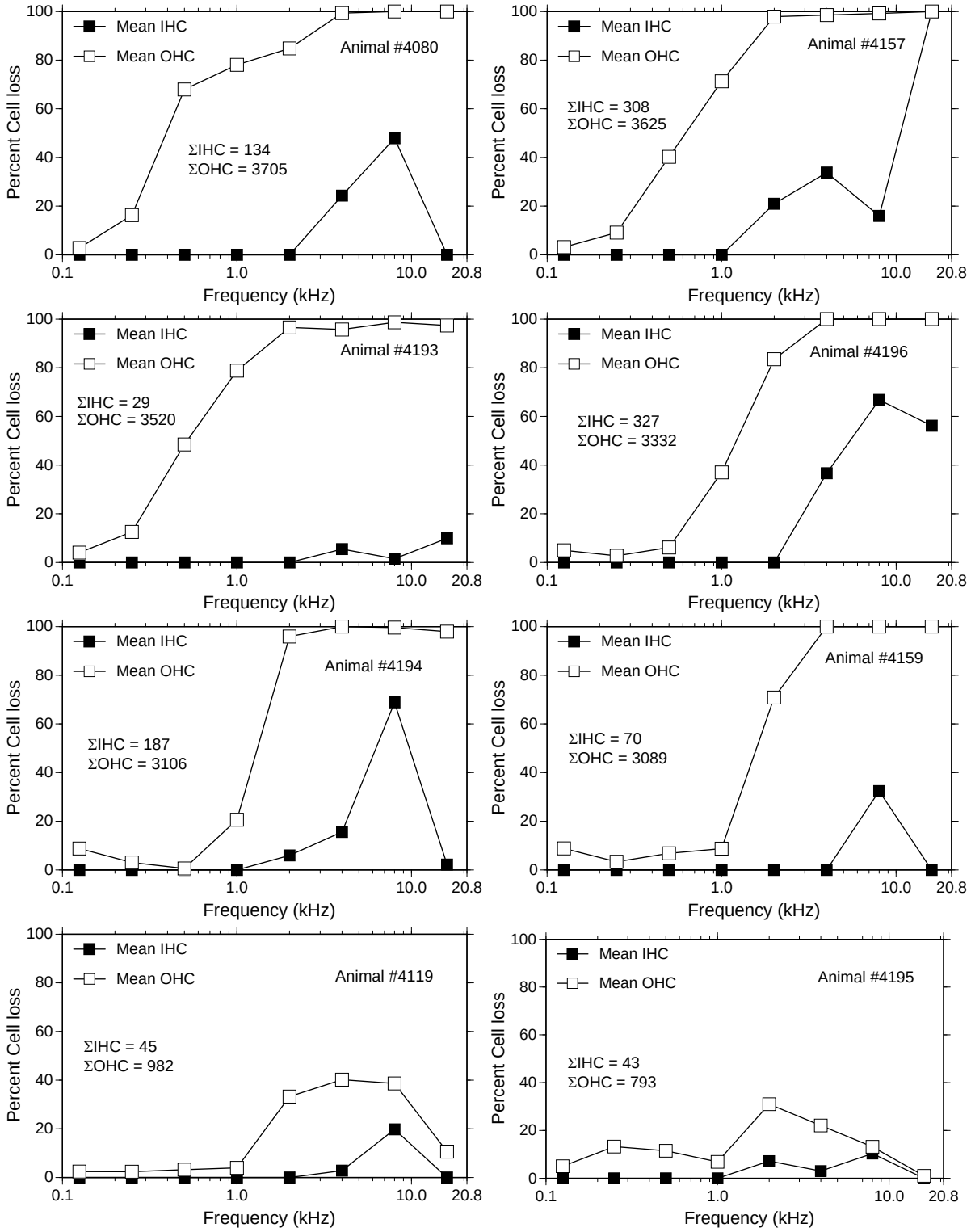


Figure 56. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours (noise only control group). The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

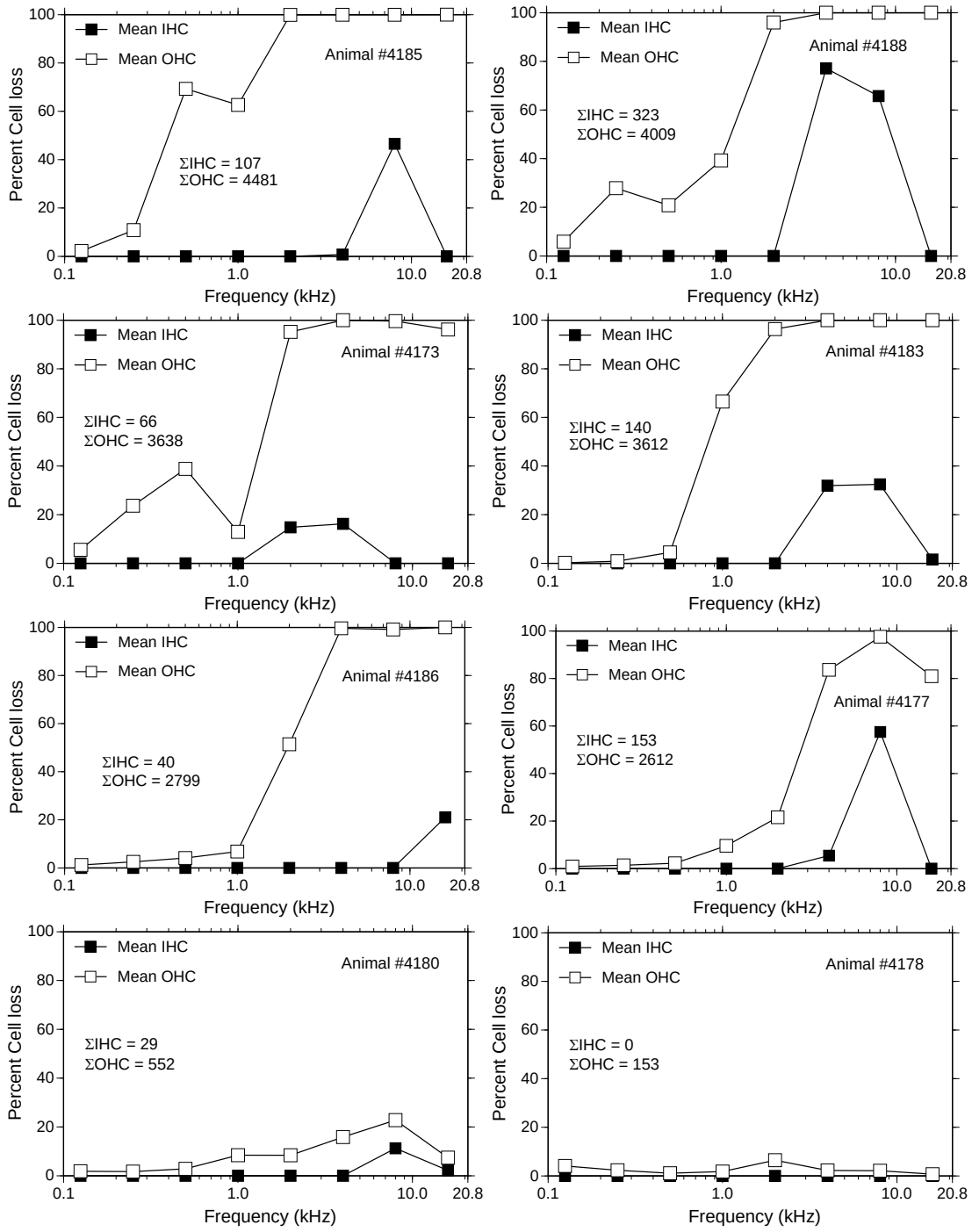


Figure 57. Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with saline injections BID for five days. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

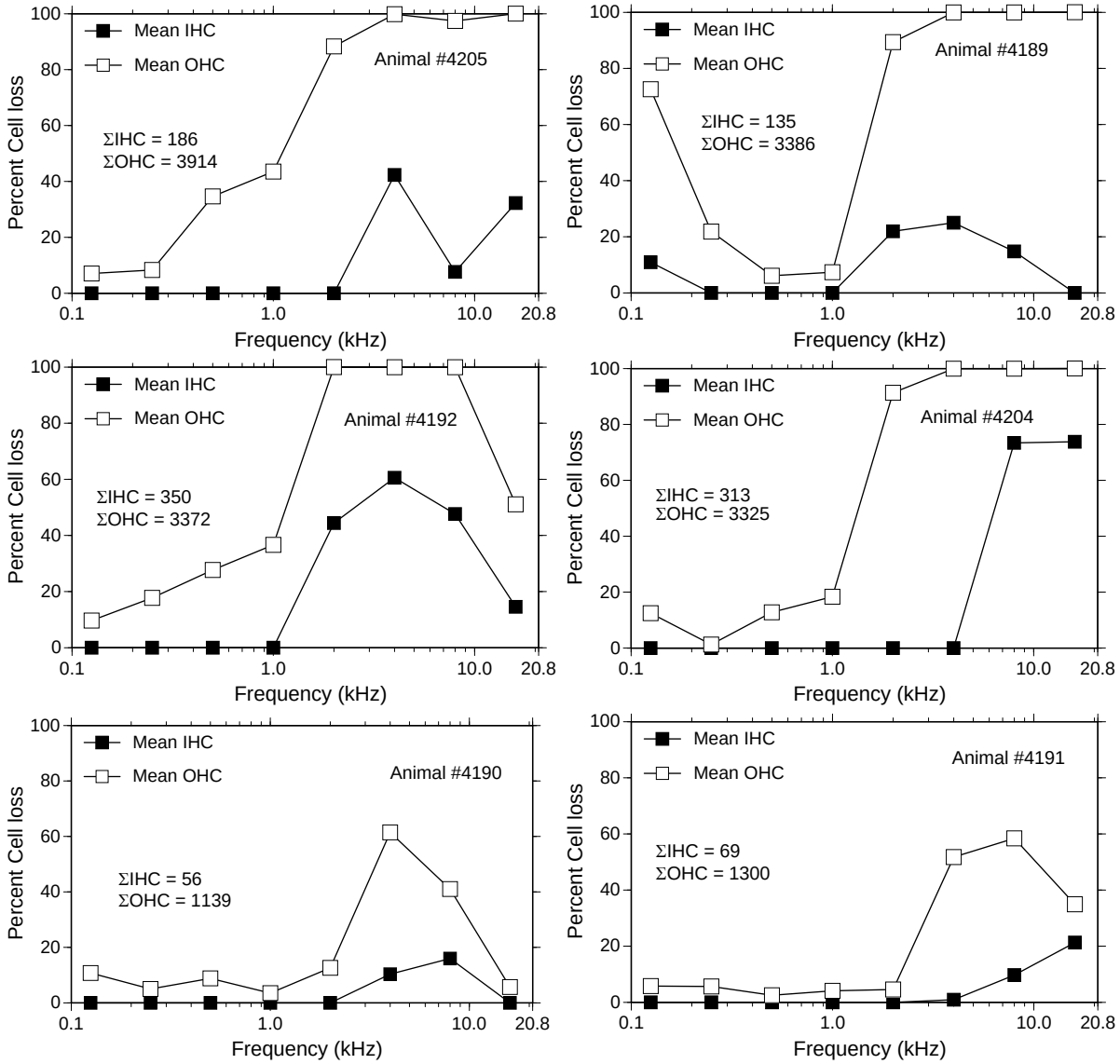


Figure 58. Individual cochleograms for the 6 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with EDTA injections BID for five days. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

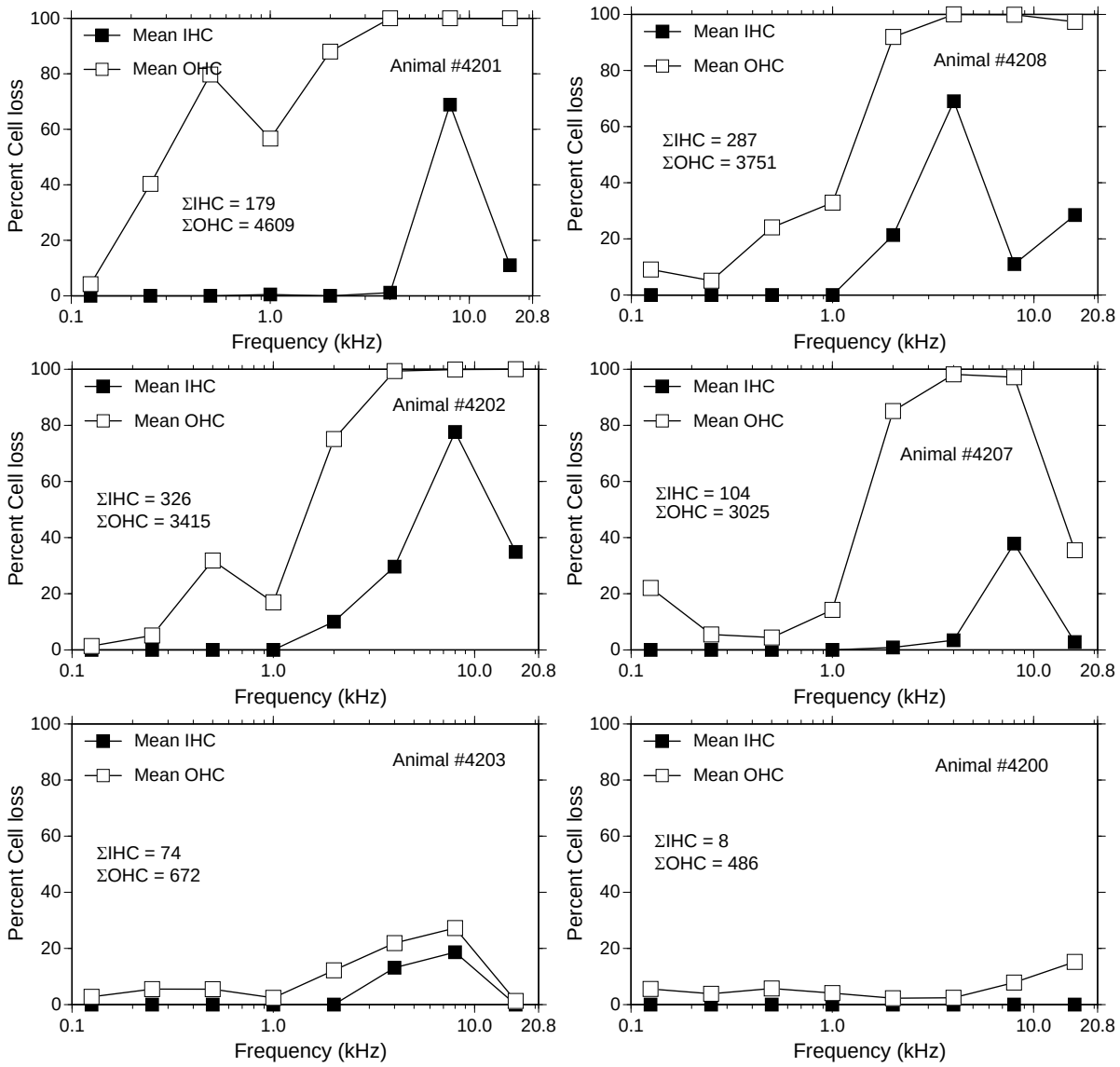


Figure 59. Individual cochleograms for the 6 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with saline plus DMSO injections BID for five days. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days postexposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

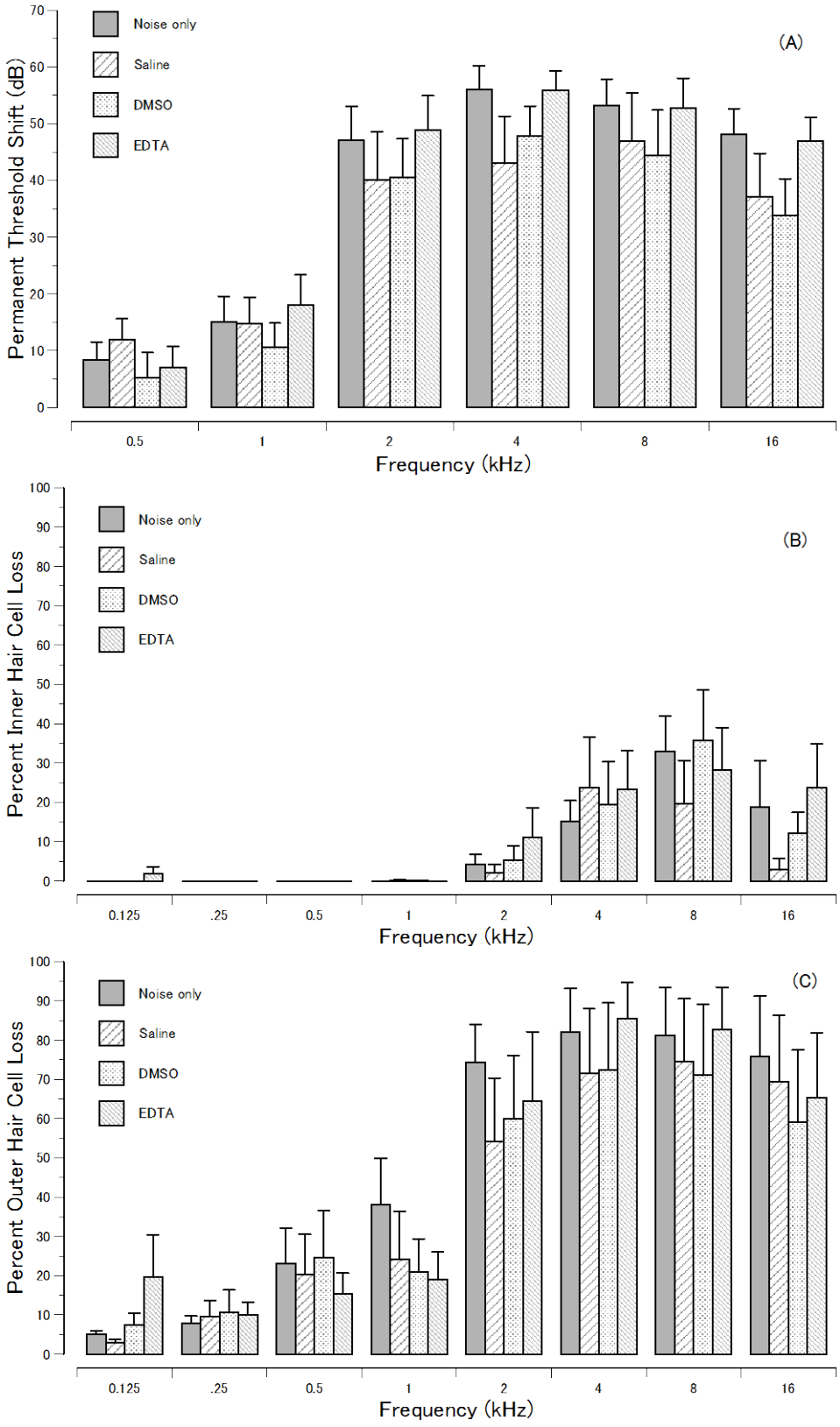


Figure 60. A comparison of the group mean PTS, %IHC and %OHC loss for the 4 control groups exposed to the 108 dB SPL, 4 kHz octave band of noise for 6 hrs. (T = standard error of the mean)

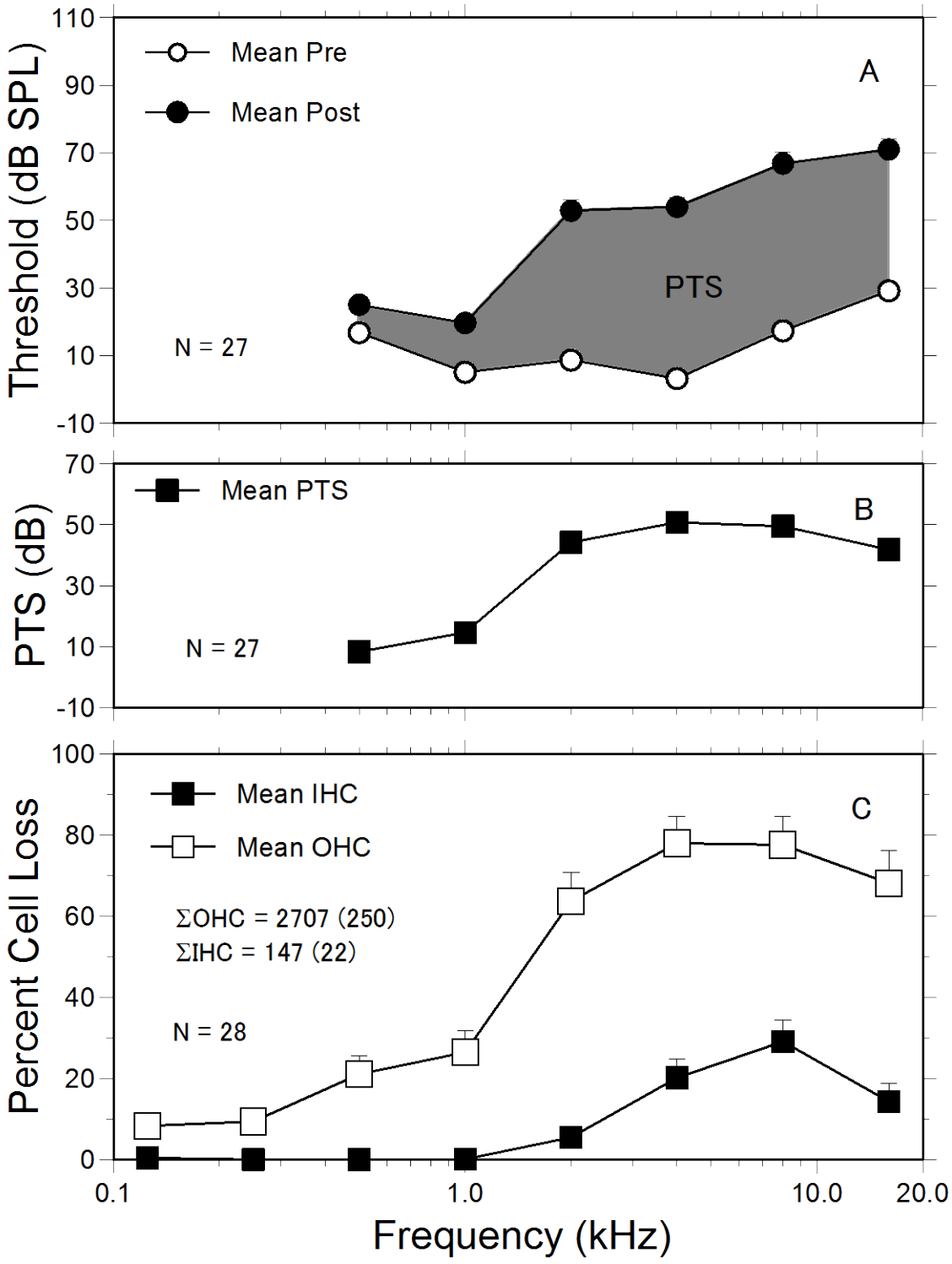


Figure 61. Phase II: Combined control group (N=28) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parantheses. (T = standard error).

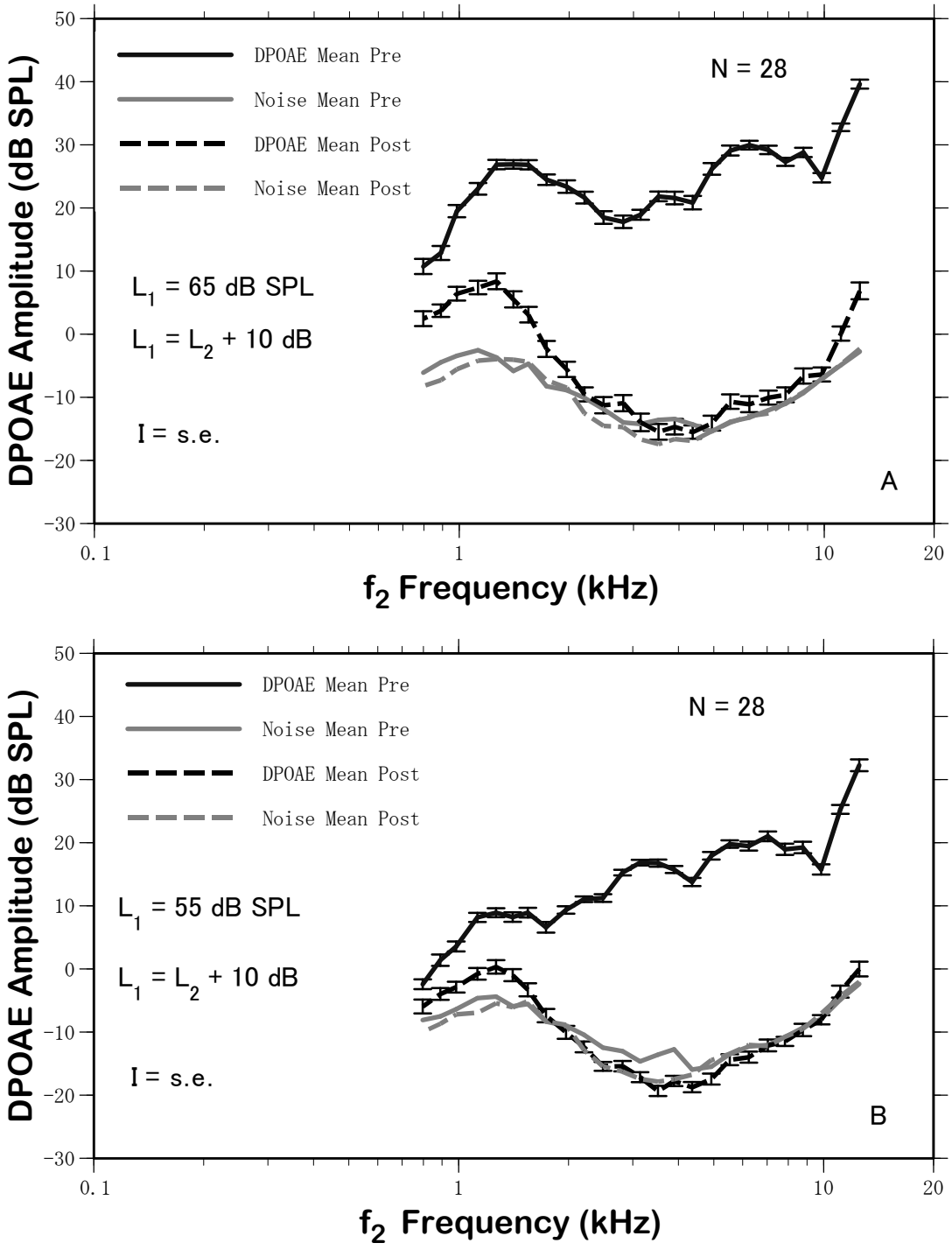


Figure 62. Phase II: Combined control group mean (N=28) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

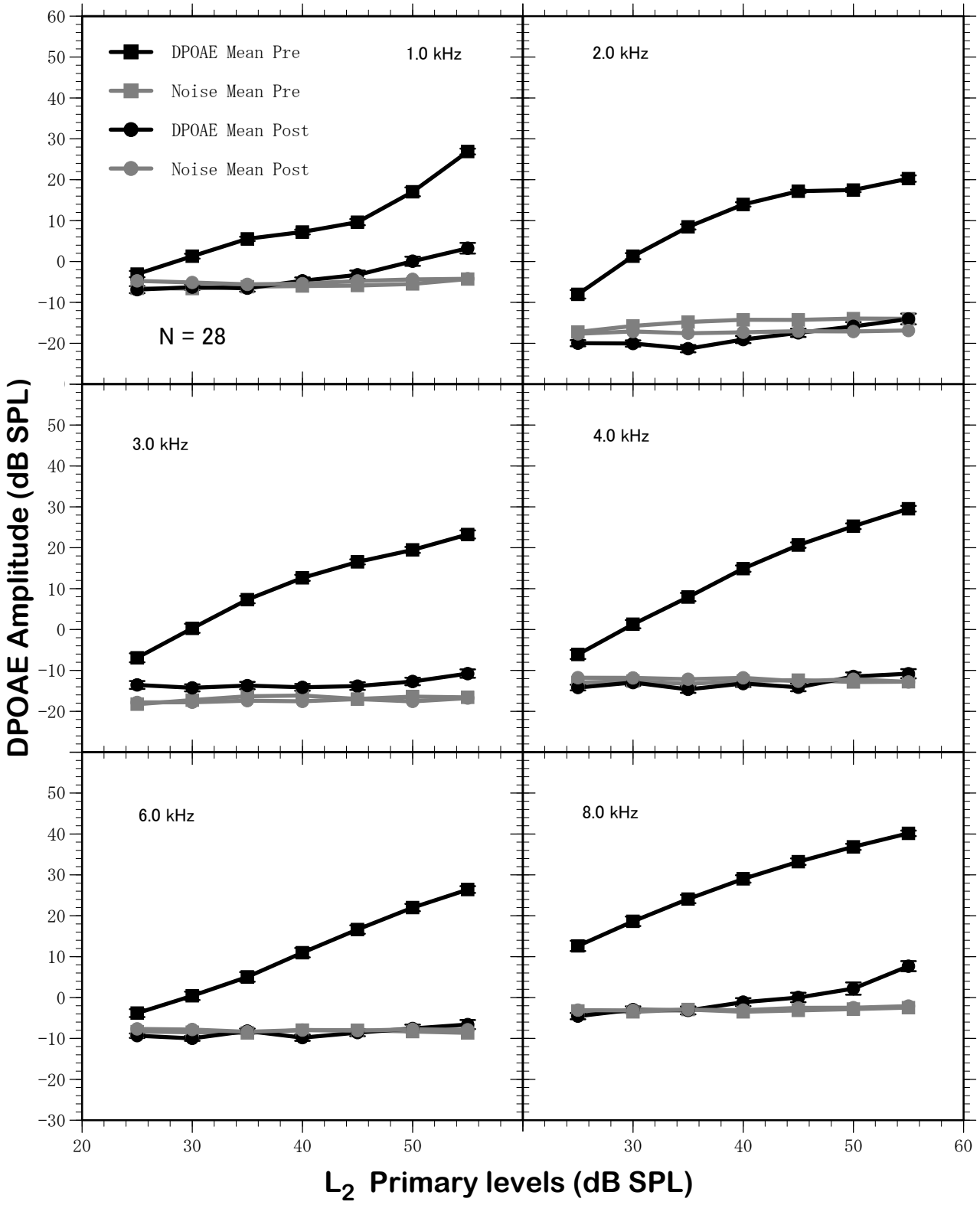


Figure 63. Phase II: Combined control group mean (N=28) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

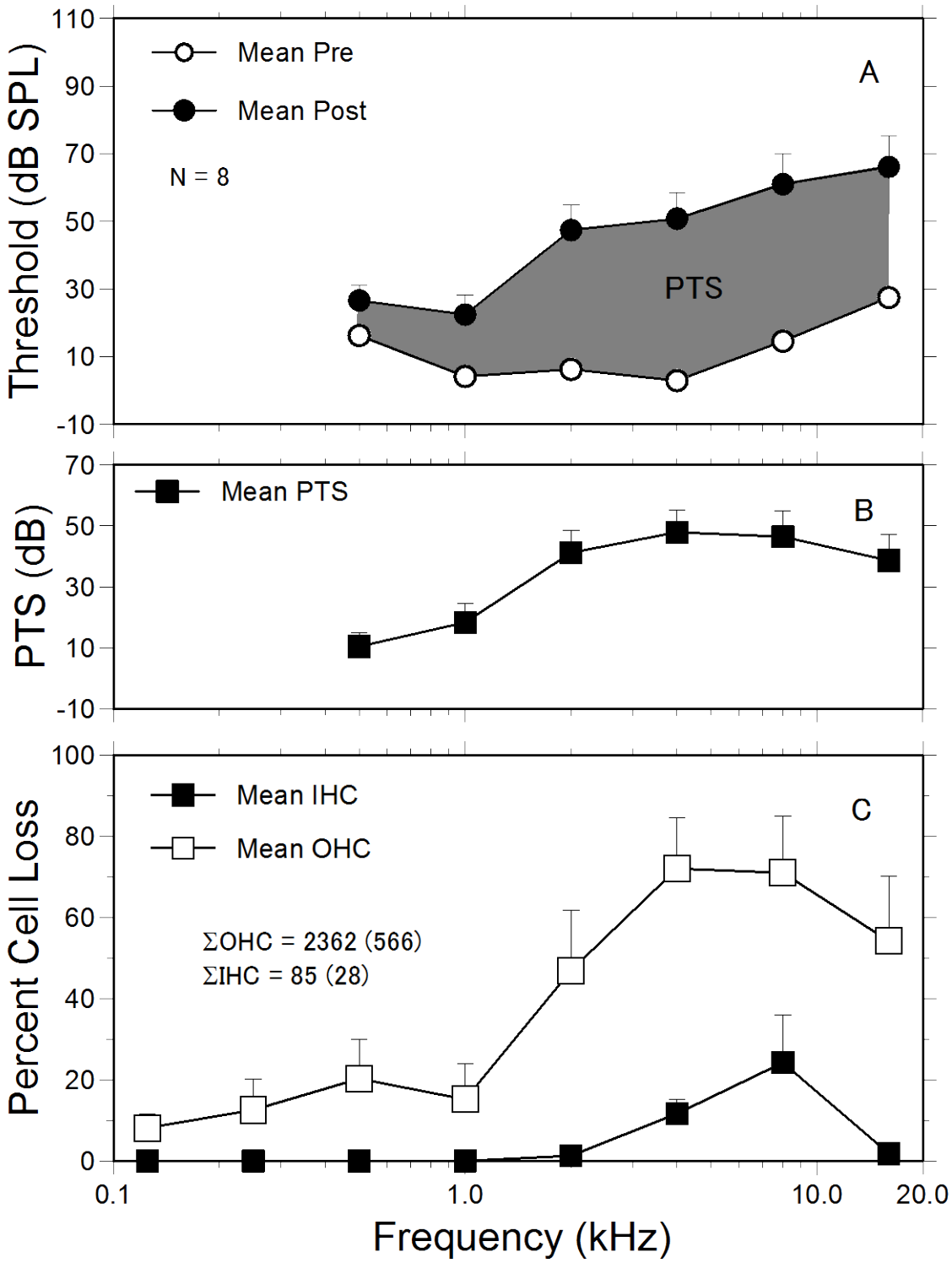


Figure 64. Phase II: L-NAC treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

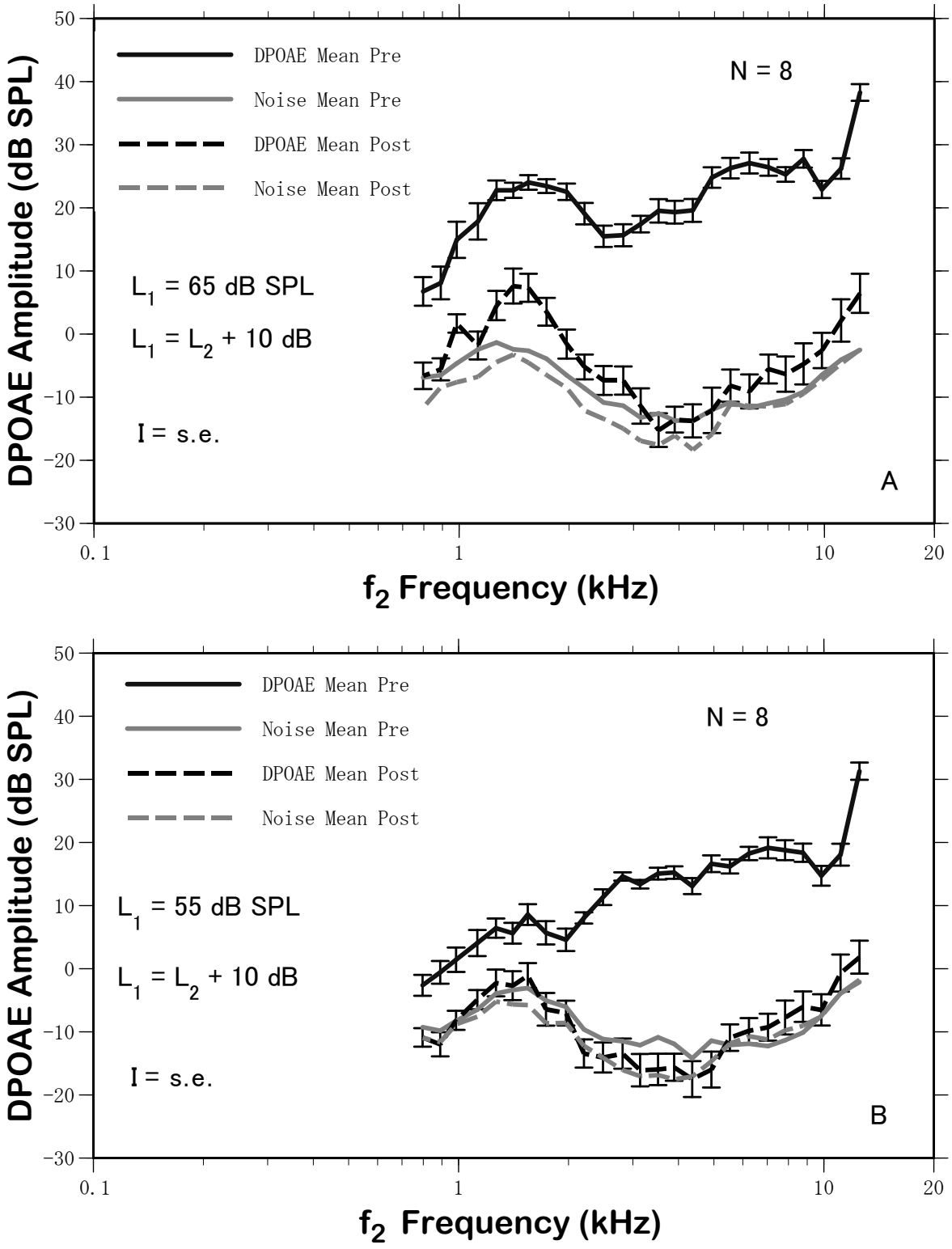


Figure 65. Phase II: L-NAC treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

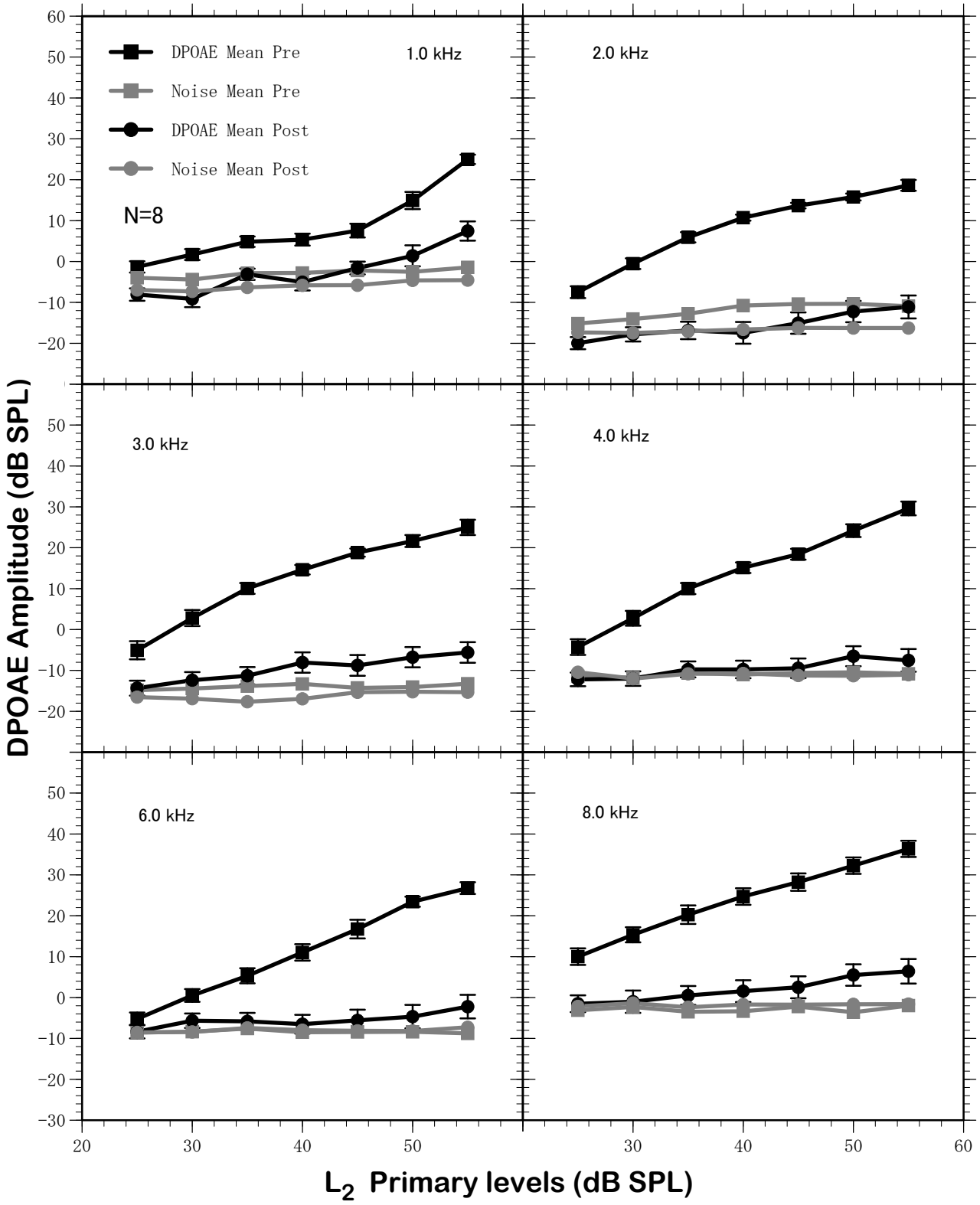


Figure 66. Phase II: L-NAC treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

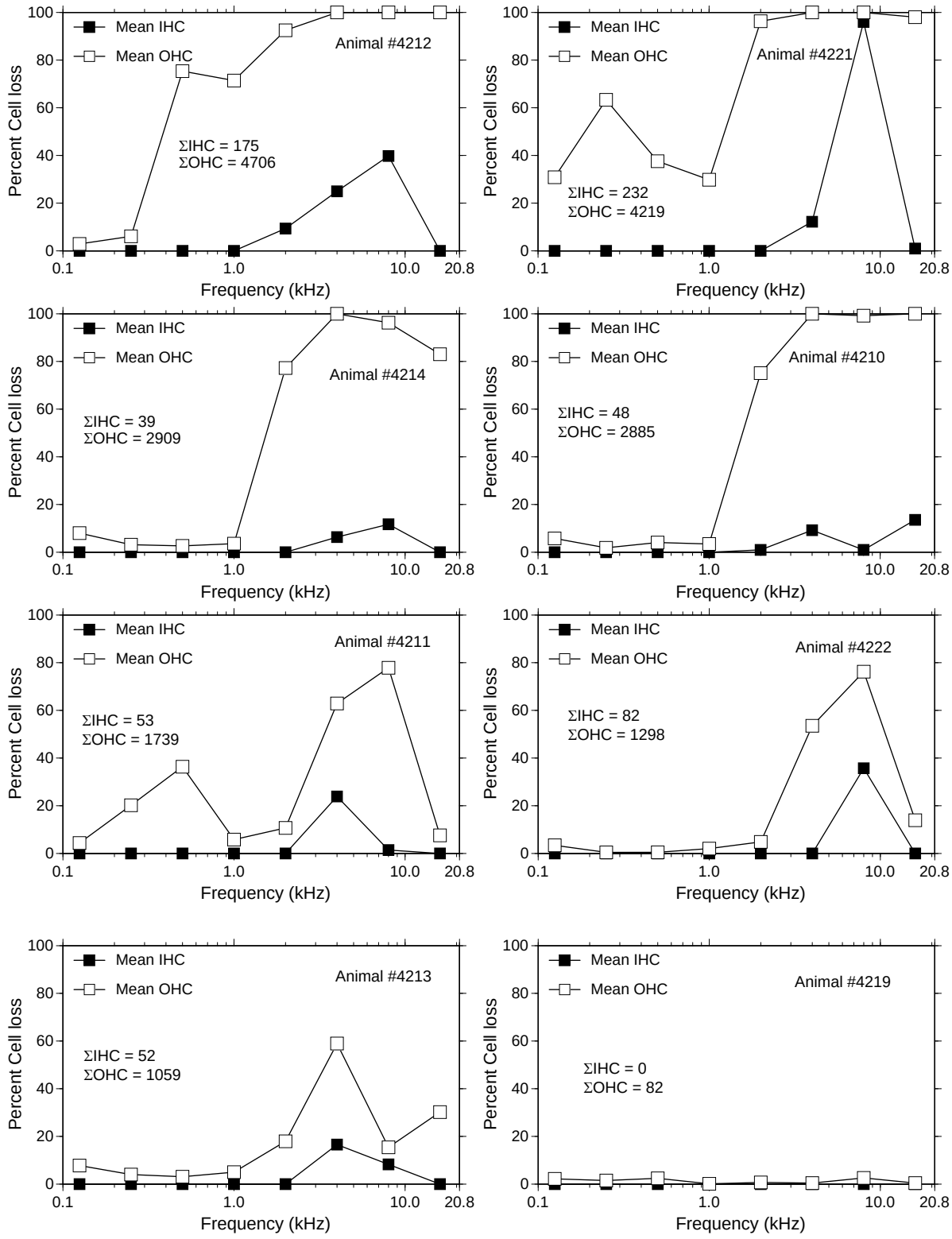


Figure 67. Phase II: Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with L-NAC injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

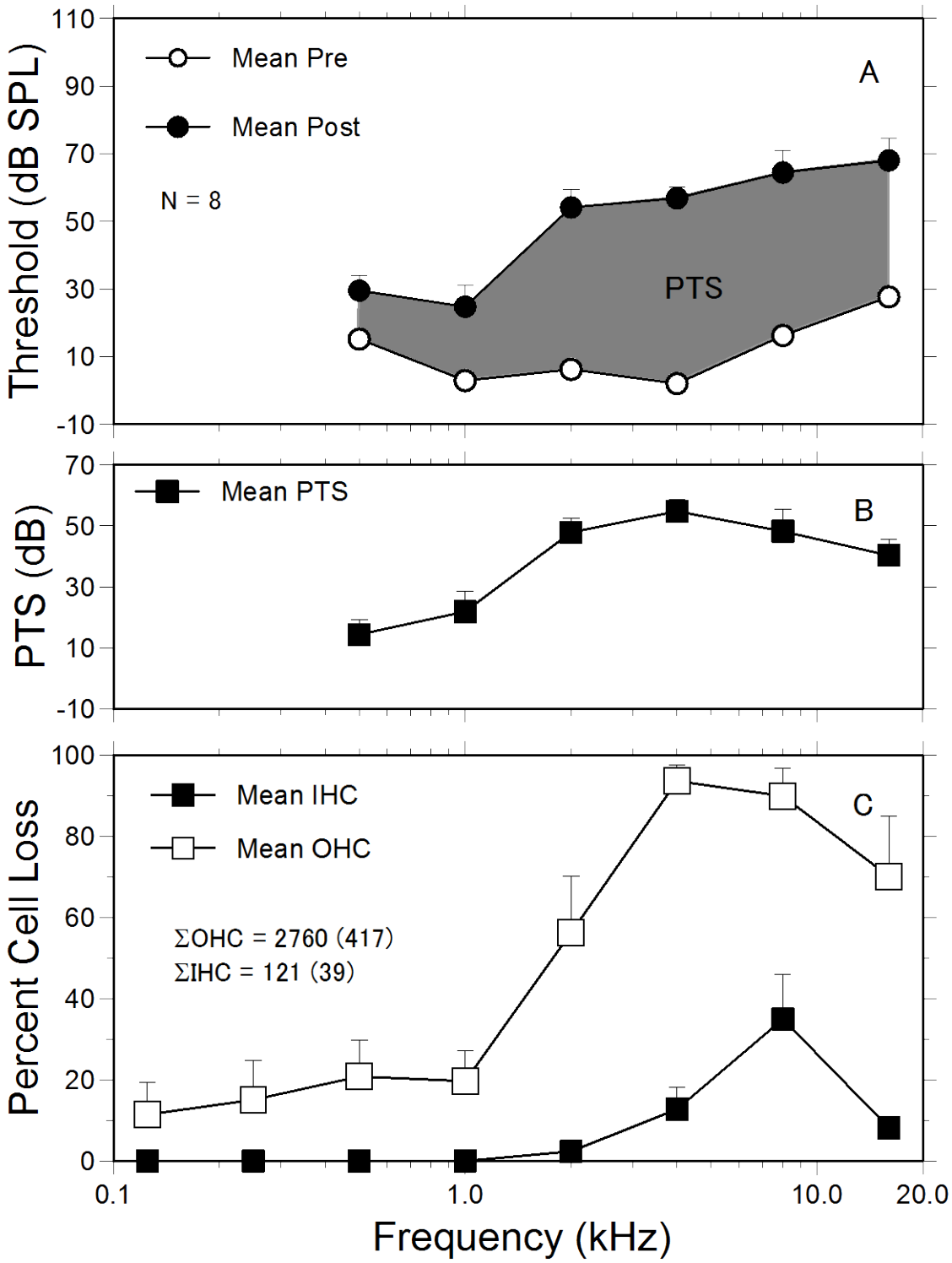


Figure 68. Phase II: ALCAR treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

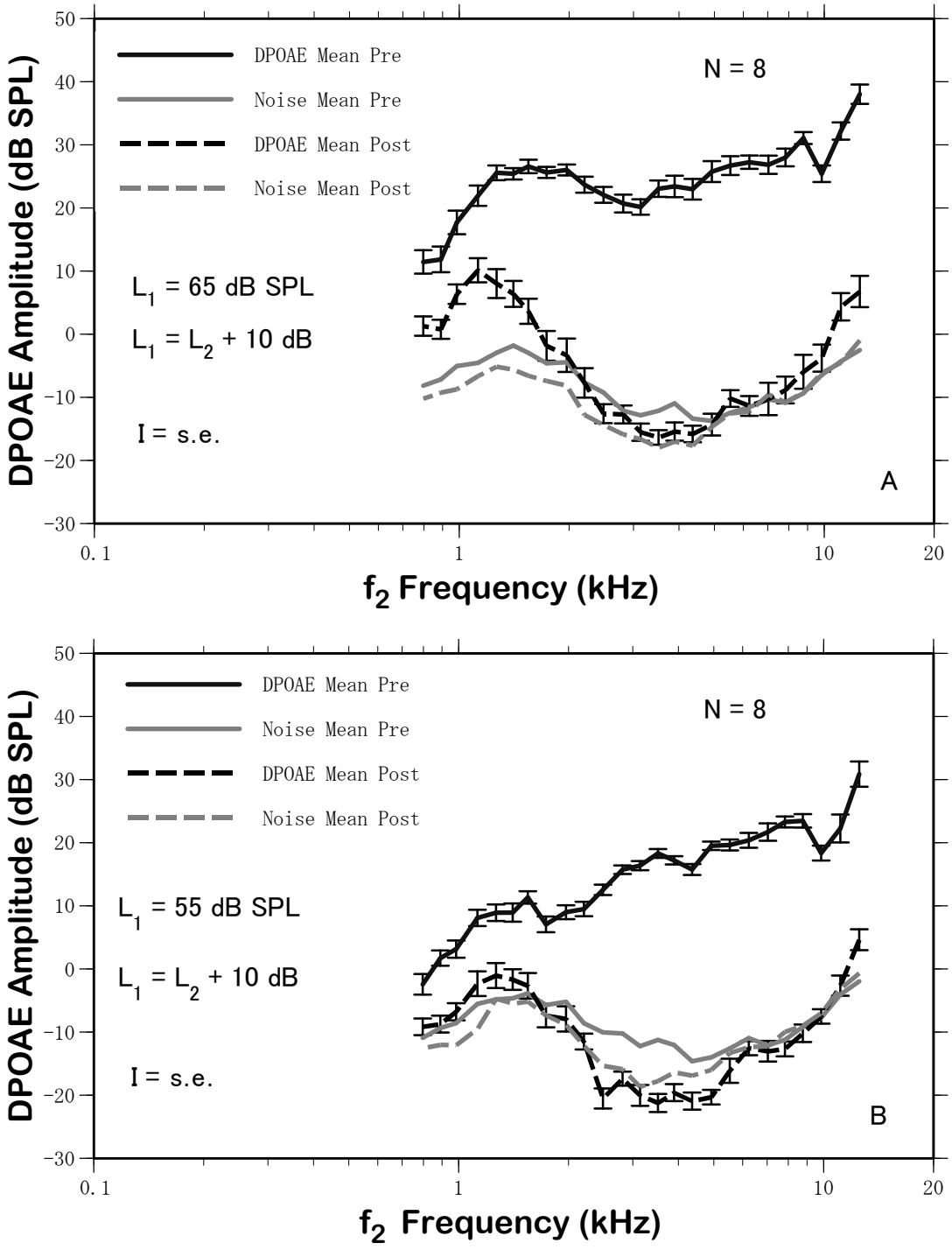


Figure 69. Phase II: ALCAR treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

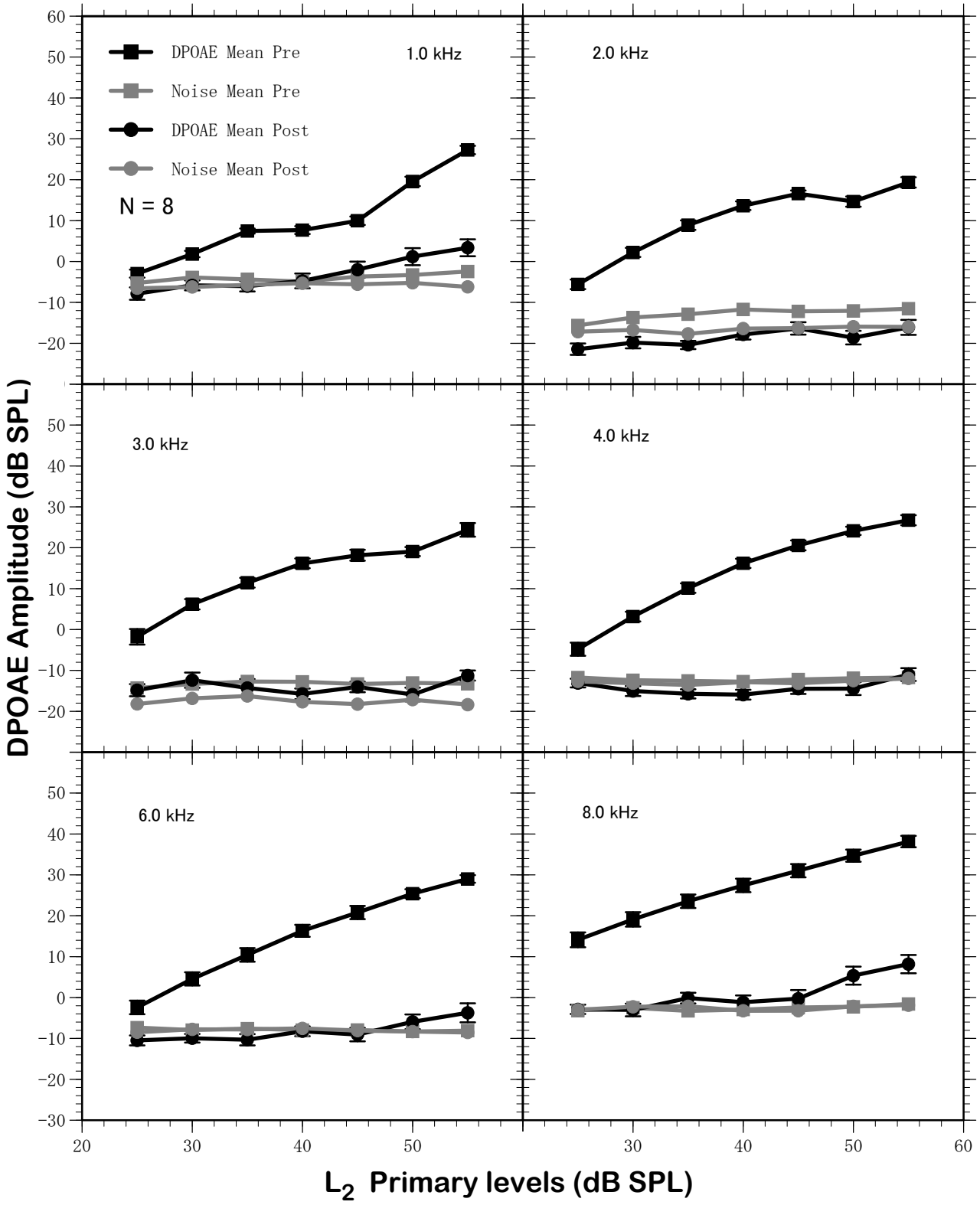


Figure 70. Phase II: ALCAR treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

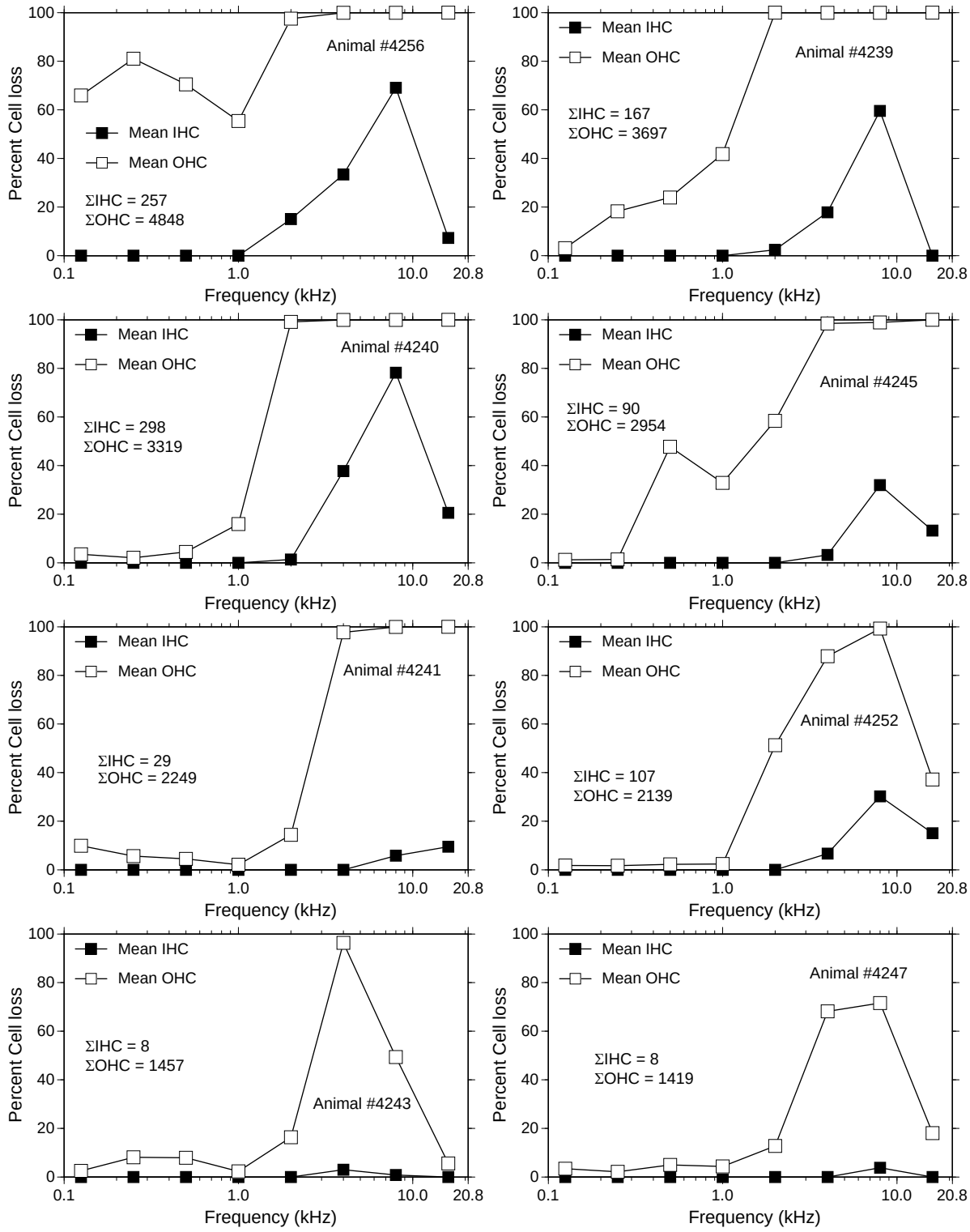


Figure 71. Phase II: Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with ALCAR injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

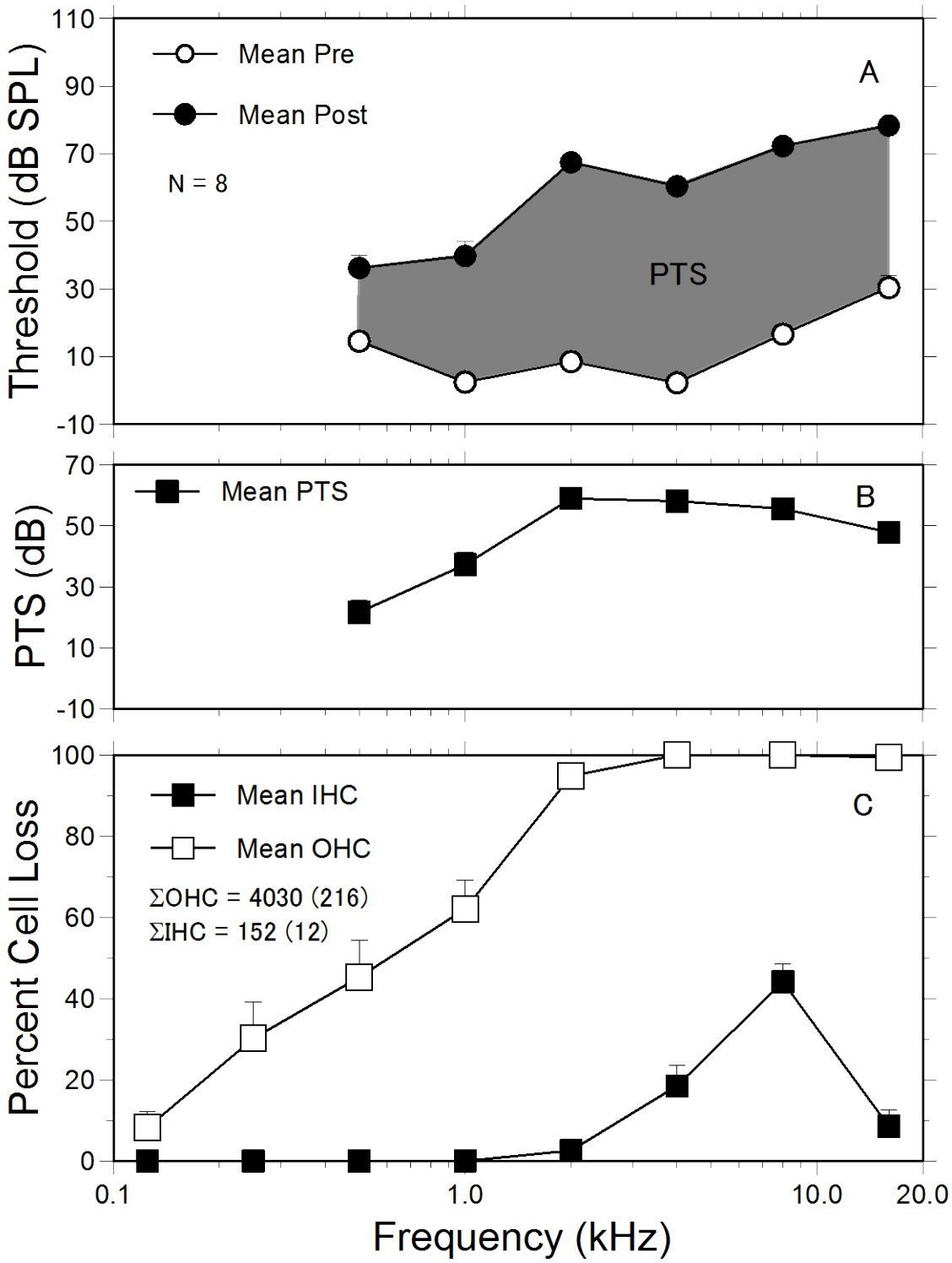


Figure 72. Phase II: D-MET treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (⊤ = standard error).

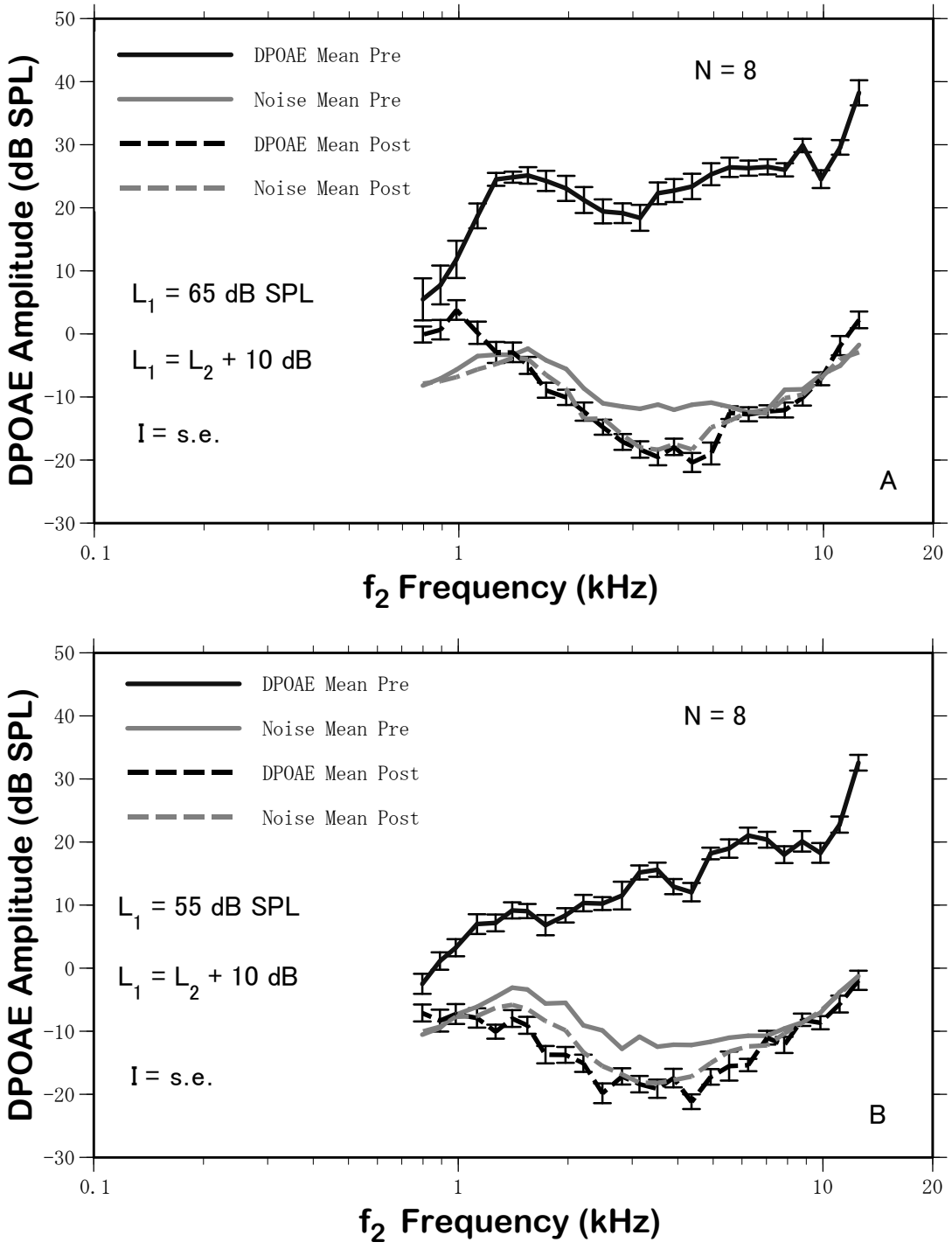


Figure 73. Phase II: D-MET treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

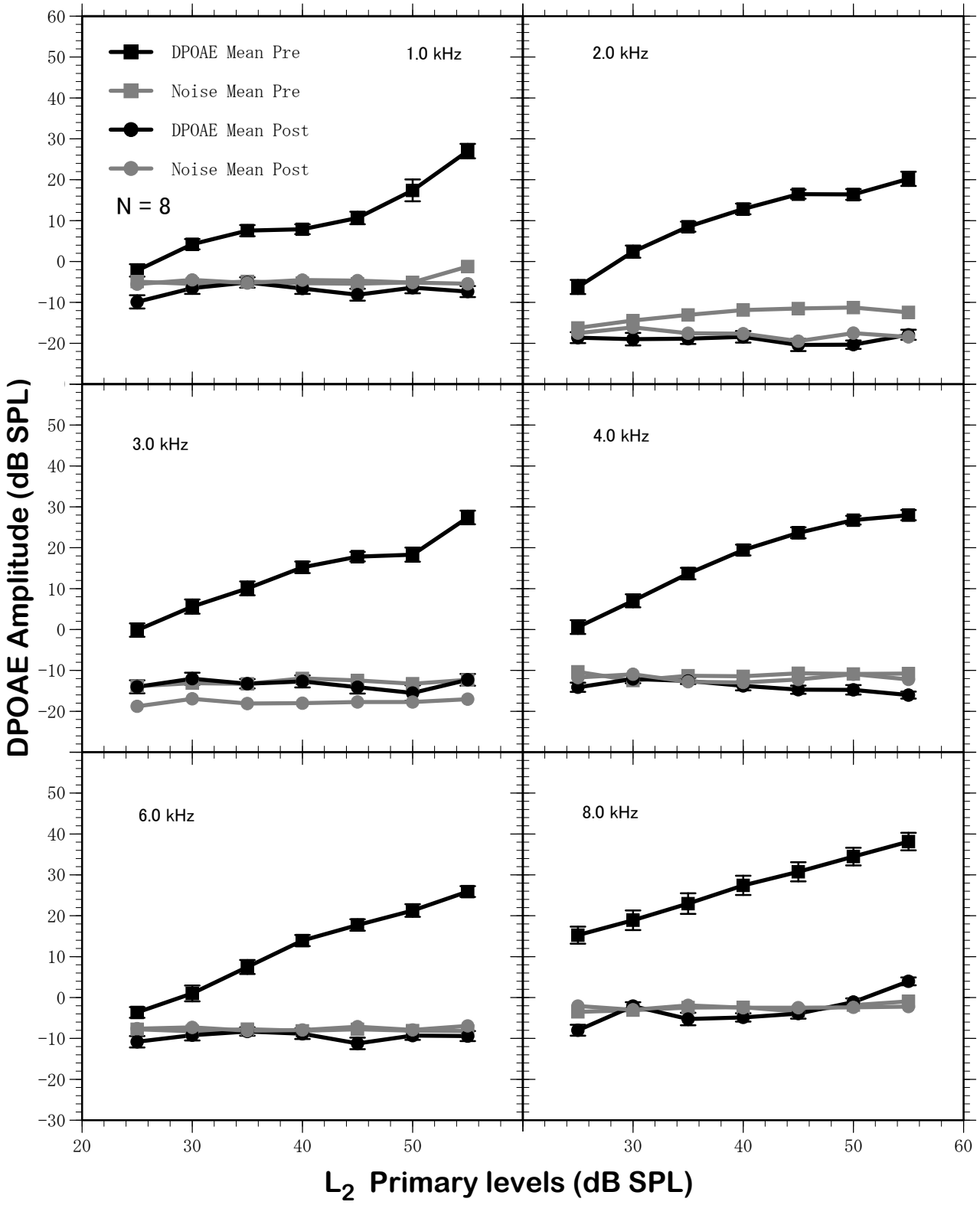


Figure 74. Phase II: D-MET treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

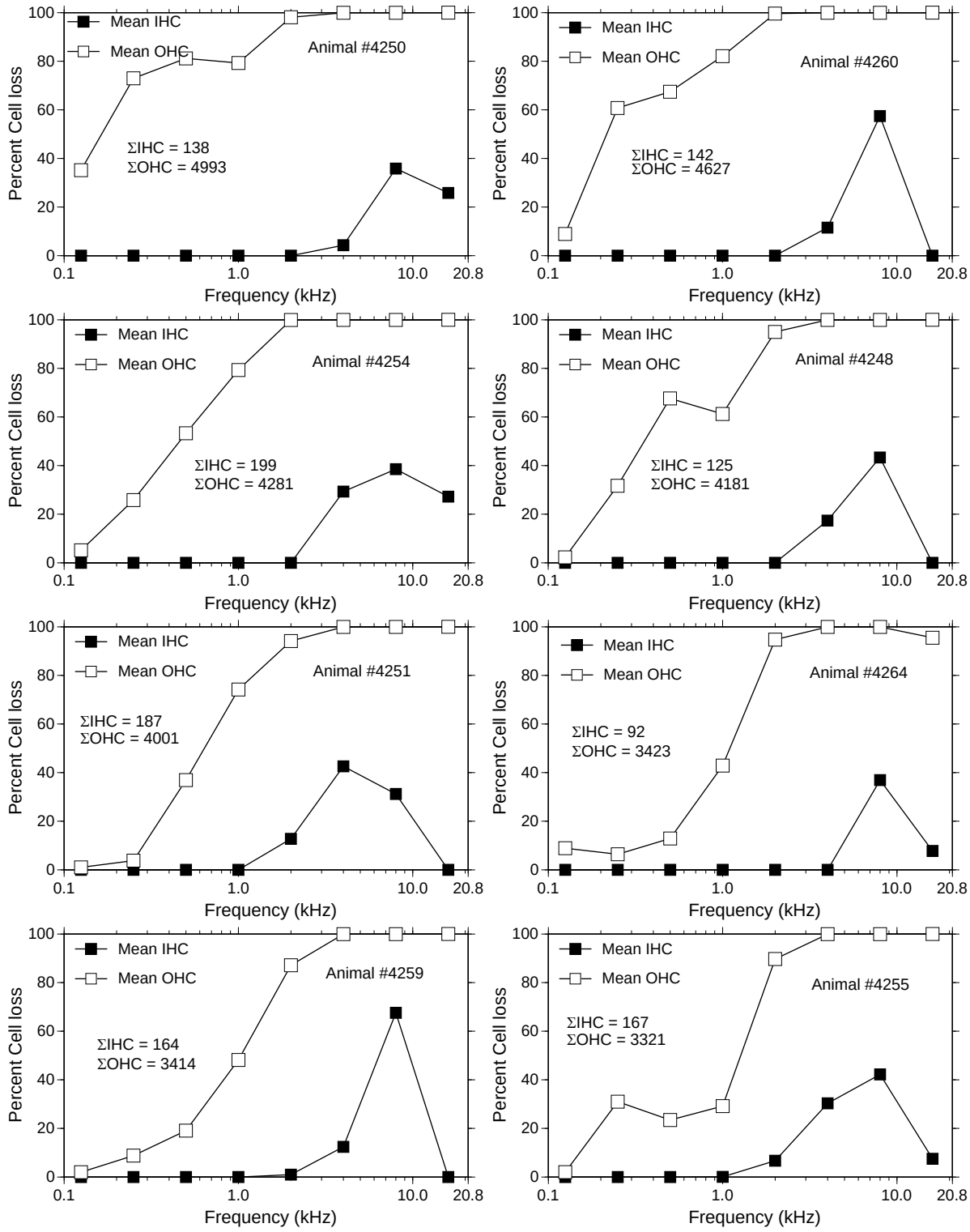


Figure 75. Phase II: Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with D-MET injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

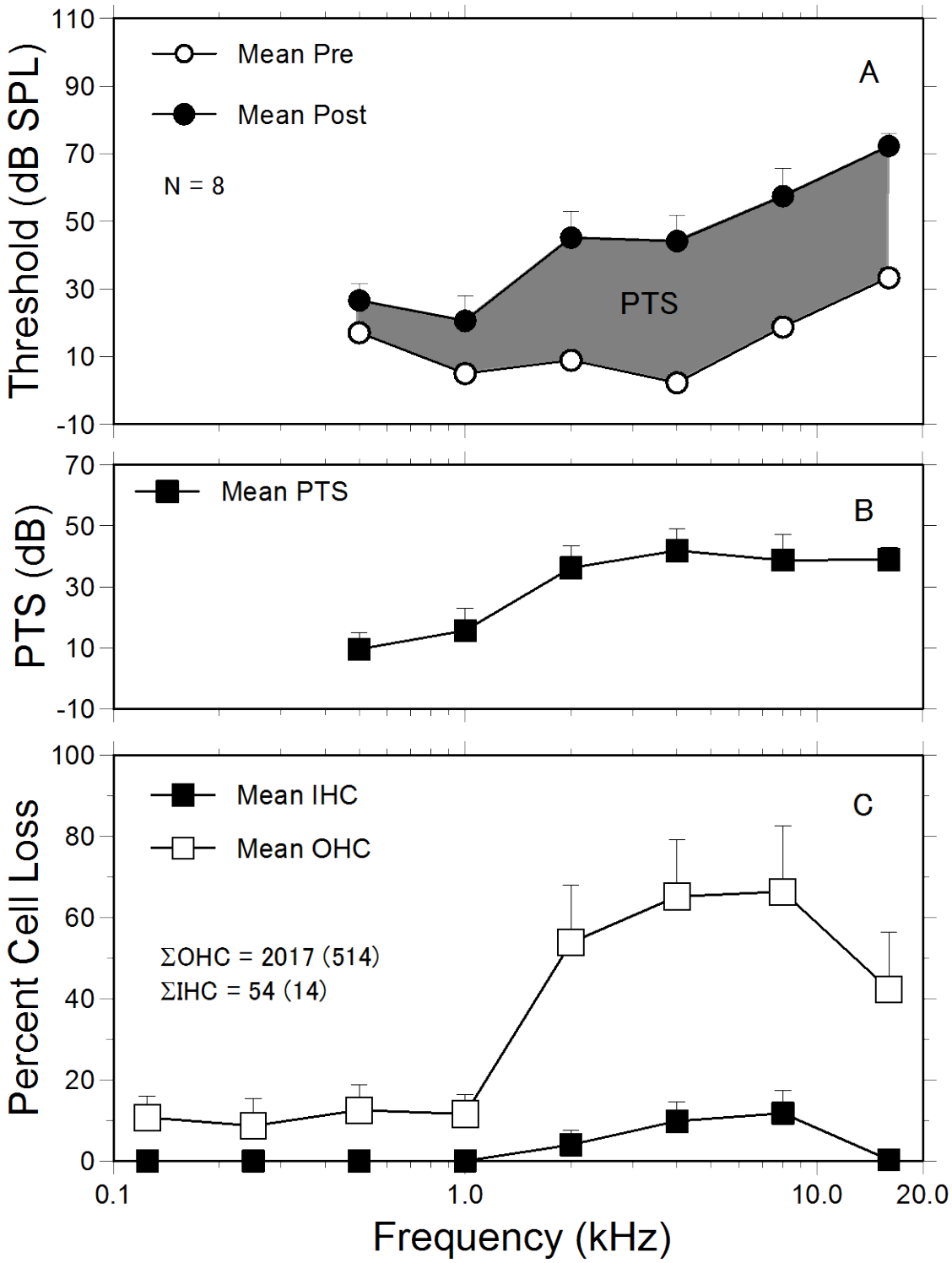


Figure 76. Phase II: Ebselen treated group (N=8) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

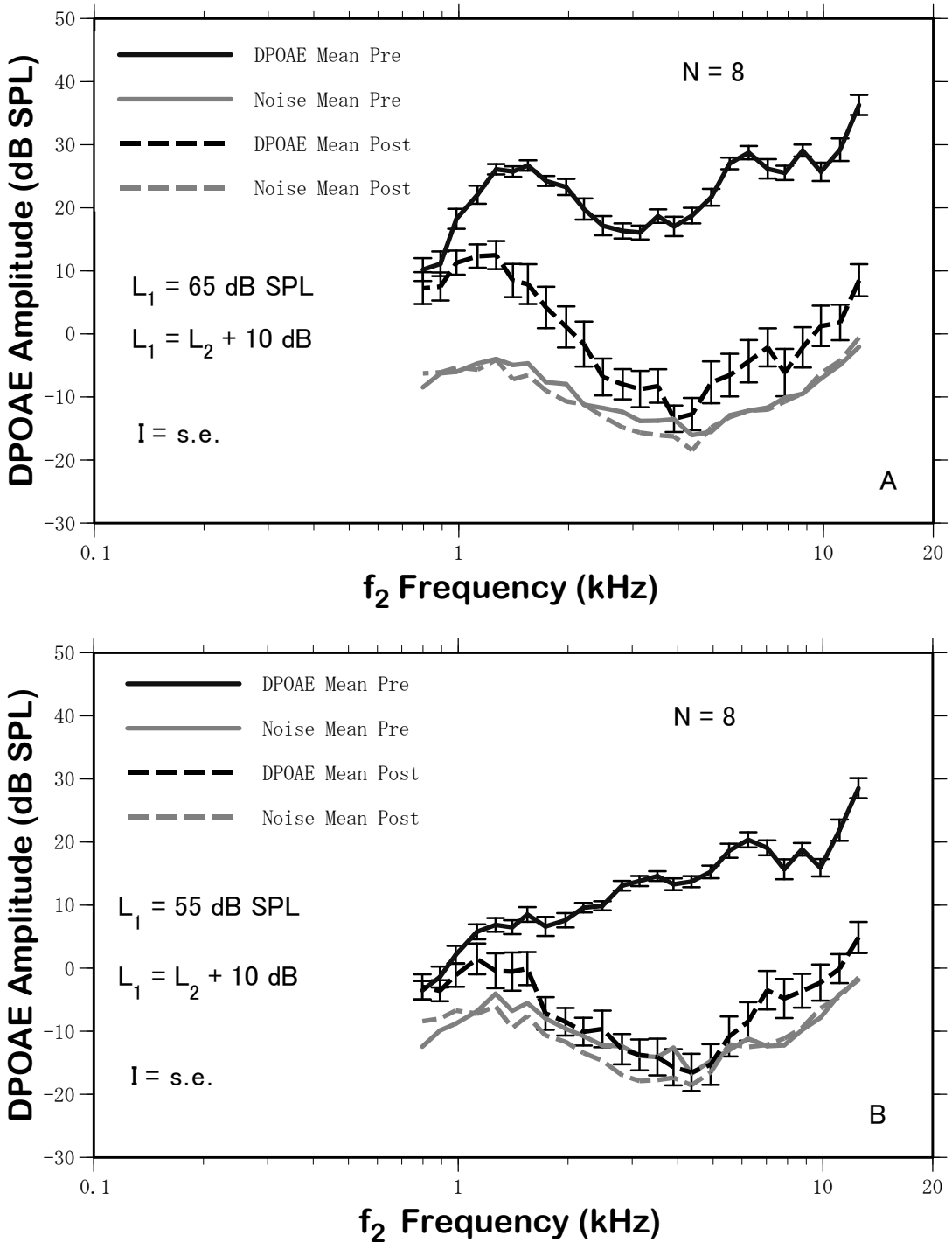


Figure 77. Phase II: Ebselen treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

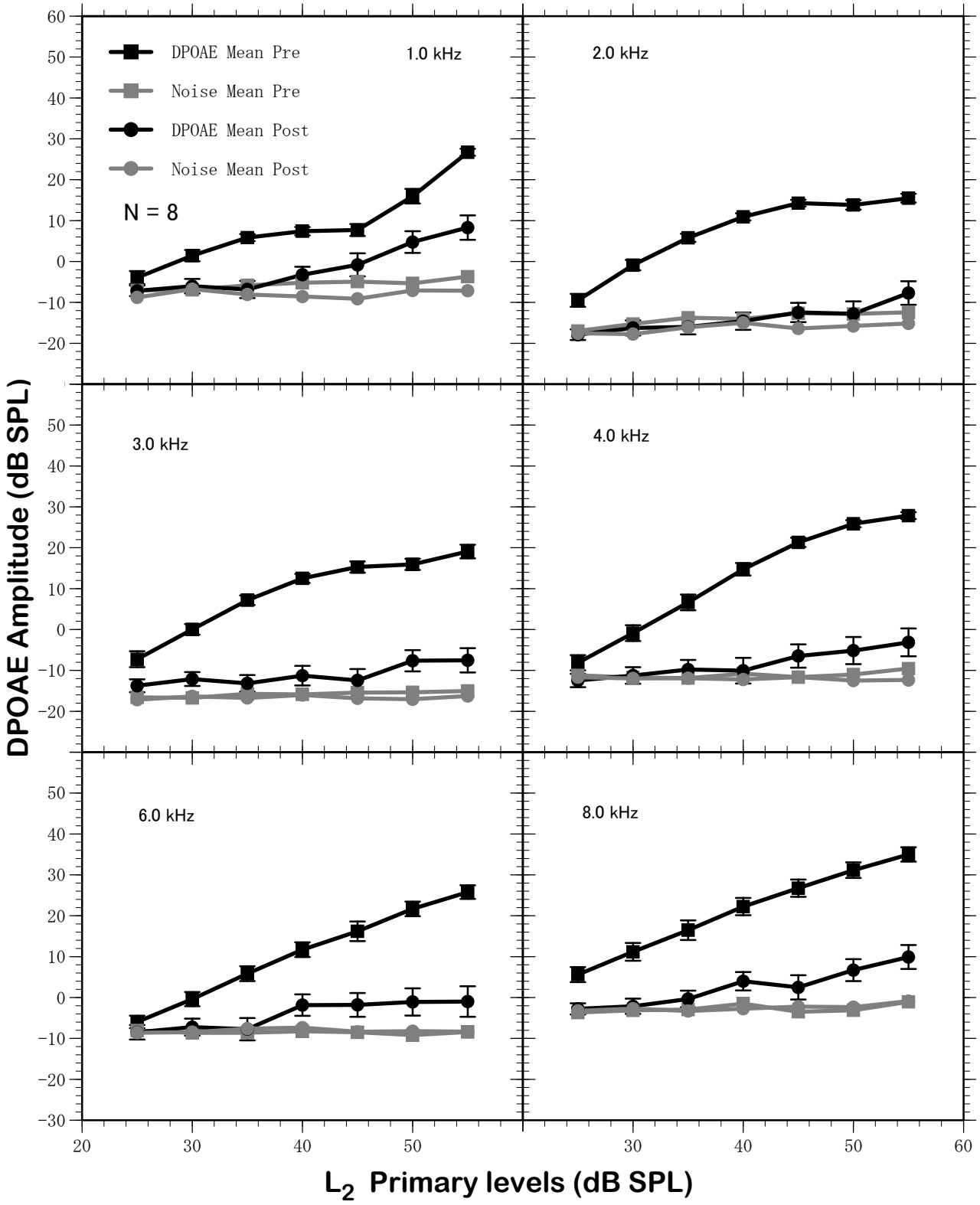


Figure 78. Phase II: Ebselen treated group mean (N=8) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

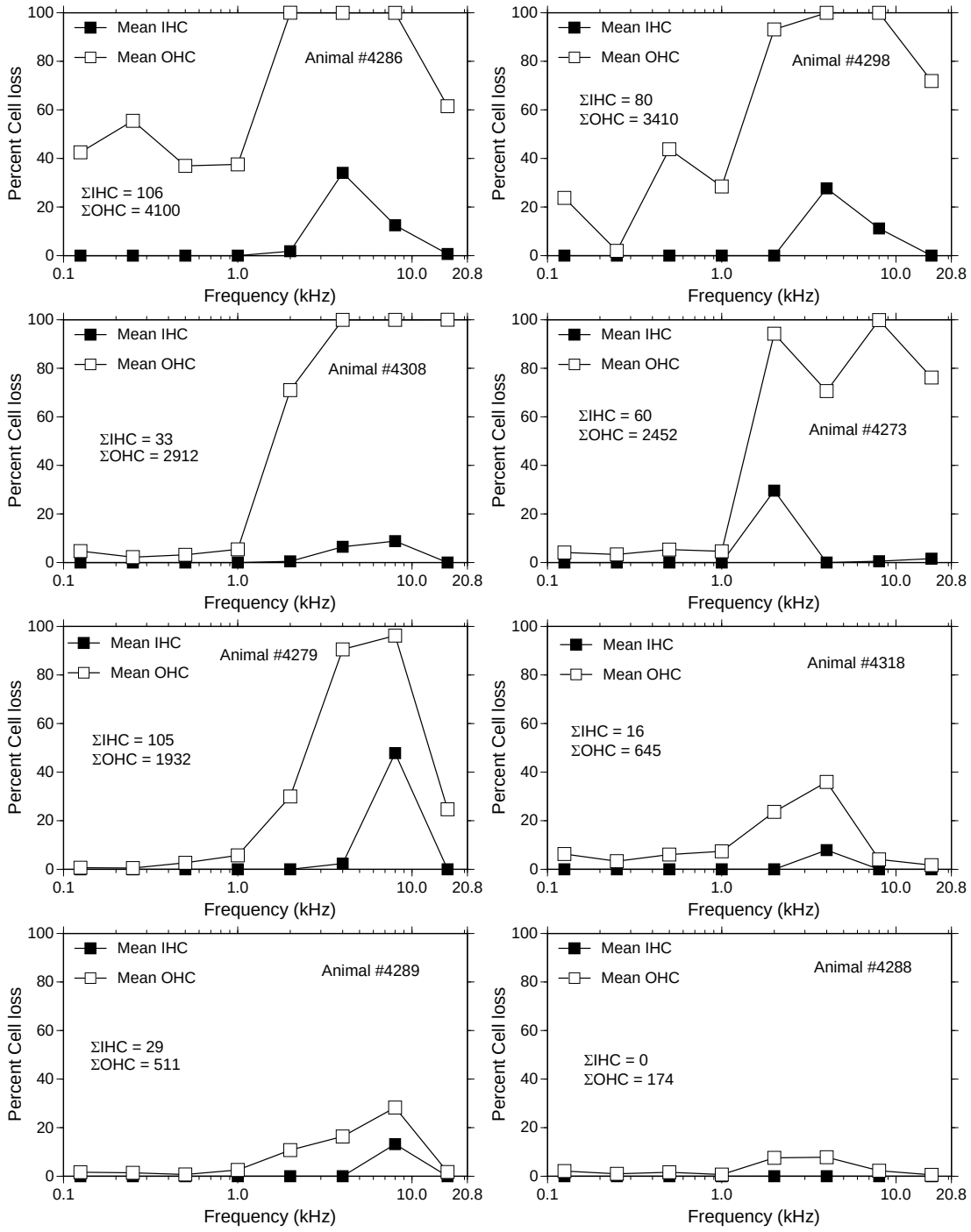


Figure 79. Phase II: Individual cochleograms for the 8 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with Ebselen injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

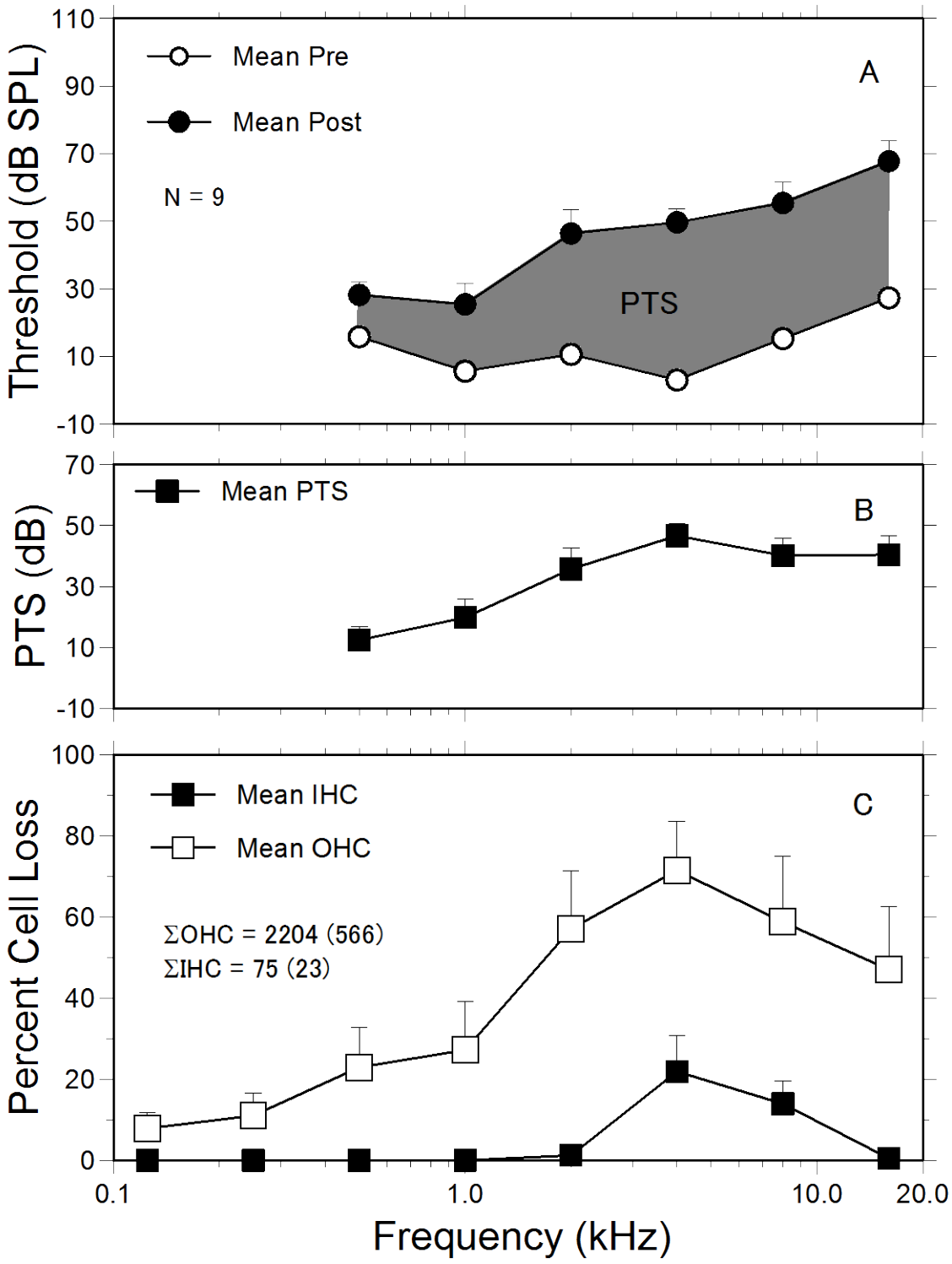


Figure 80. Phase II: Src Inh KX1-004 treated group (N=9) exposed to a 108 dB SPL, 4 kHz octave band of noise for 6 hours. (A) pre and post exposure audiograms. (B) Permanent threshold shift (PTS) and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = standard error).

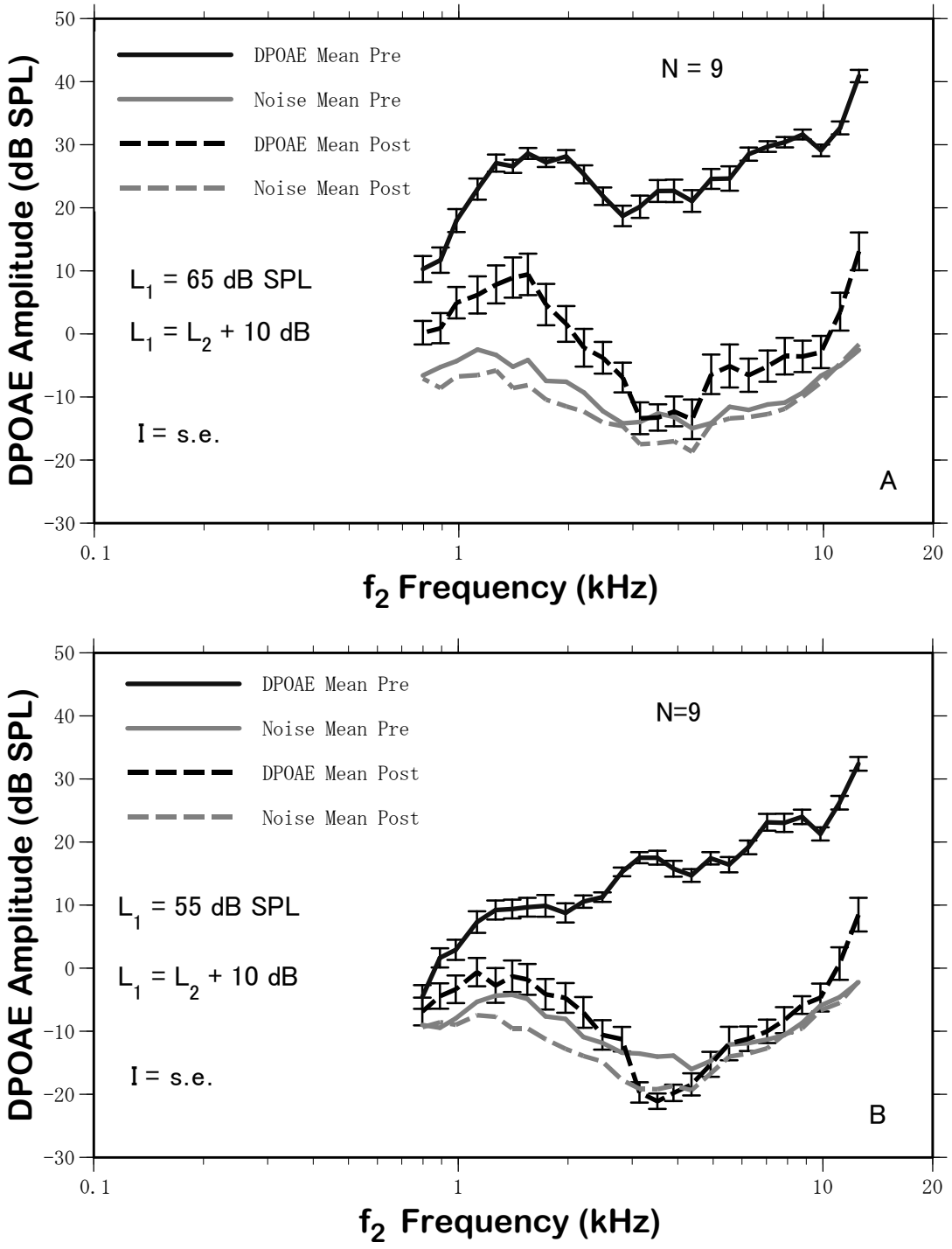


Figure 81. Phase II: Src Inh KX1-004 treated group mean (N=9) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

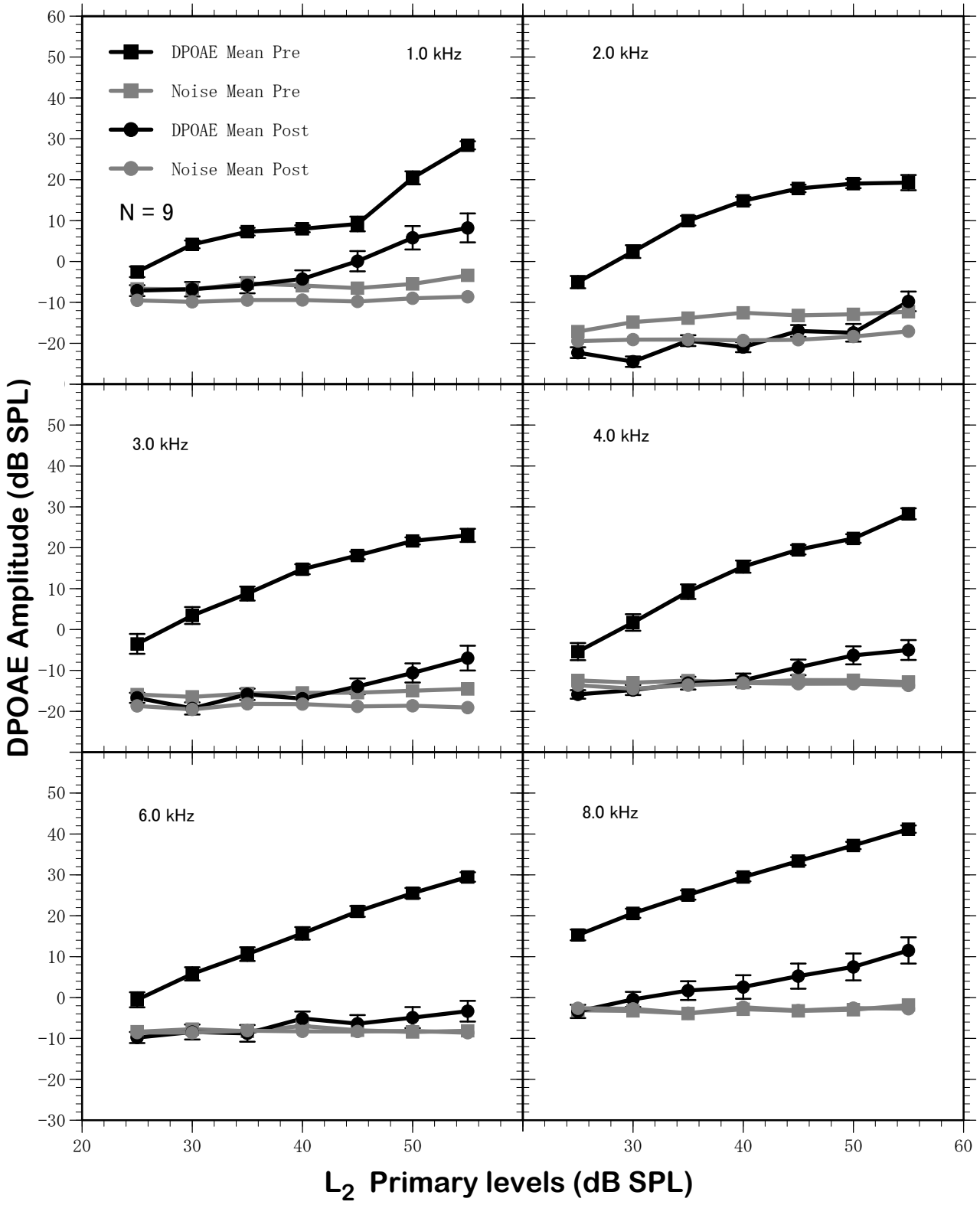


Figure 82. Phase II: Src Inh KX1-004 treated group mean (N=9) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 4 kHz, octave band of noise at 108 dB SPL for 6 hours. (I = s.e.)

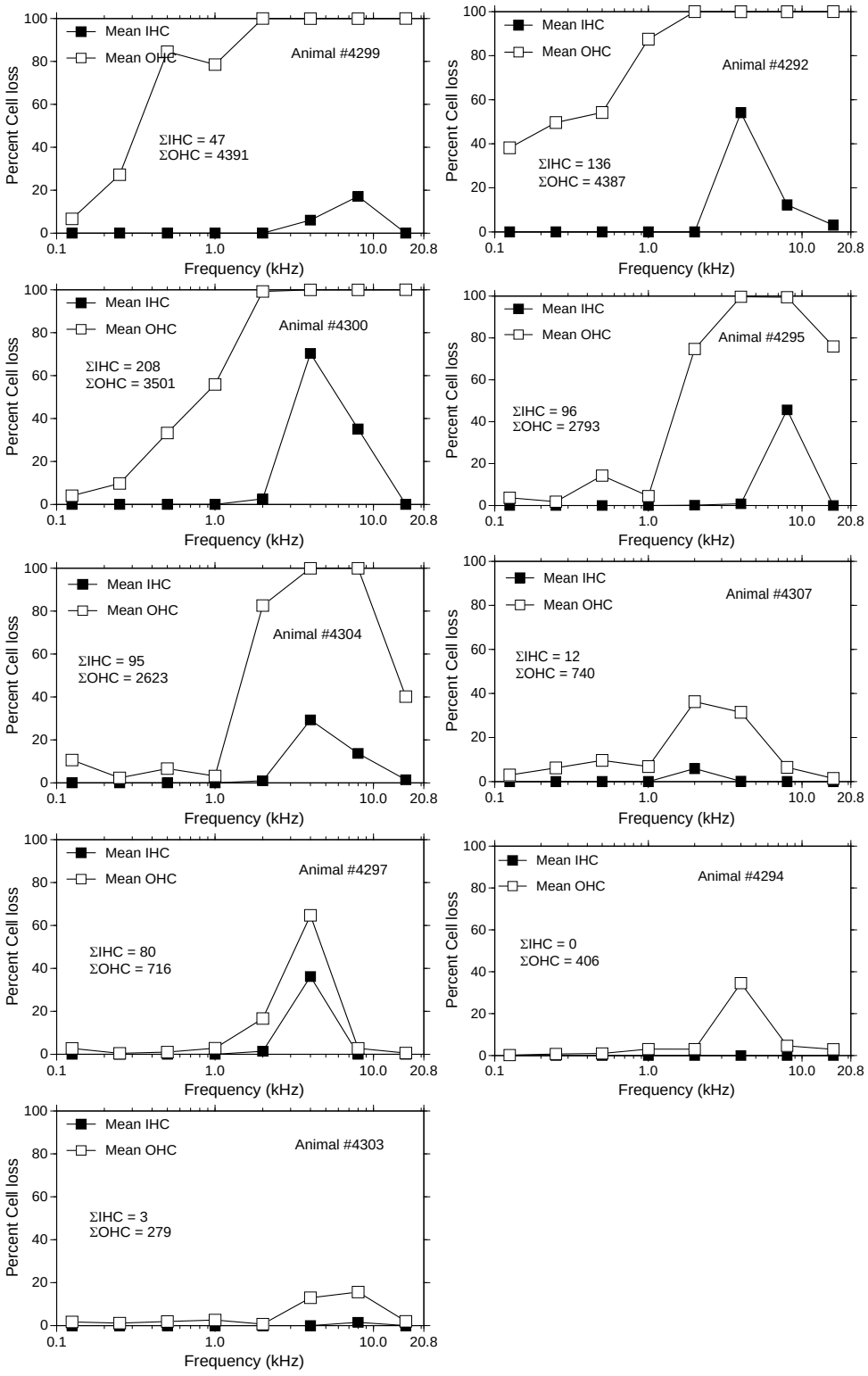


Figure 83. Phase II: Individual cochleograms for the 9 chinchillas exposed to 108 dB SPL, 4 kHz octave band noise for 6 hours and treated with Src Inh KX1-004 injections BID for five days post exposure. The panels are arranged in a descending order of severity based on the total number of outer hair cells lost. The animals were euthanized 30 days post exposure. ΣIHC and ΣOHC = total number of missing inner and outer hair cells.

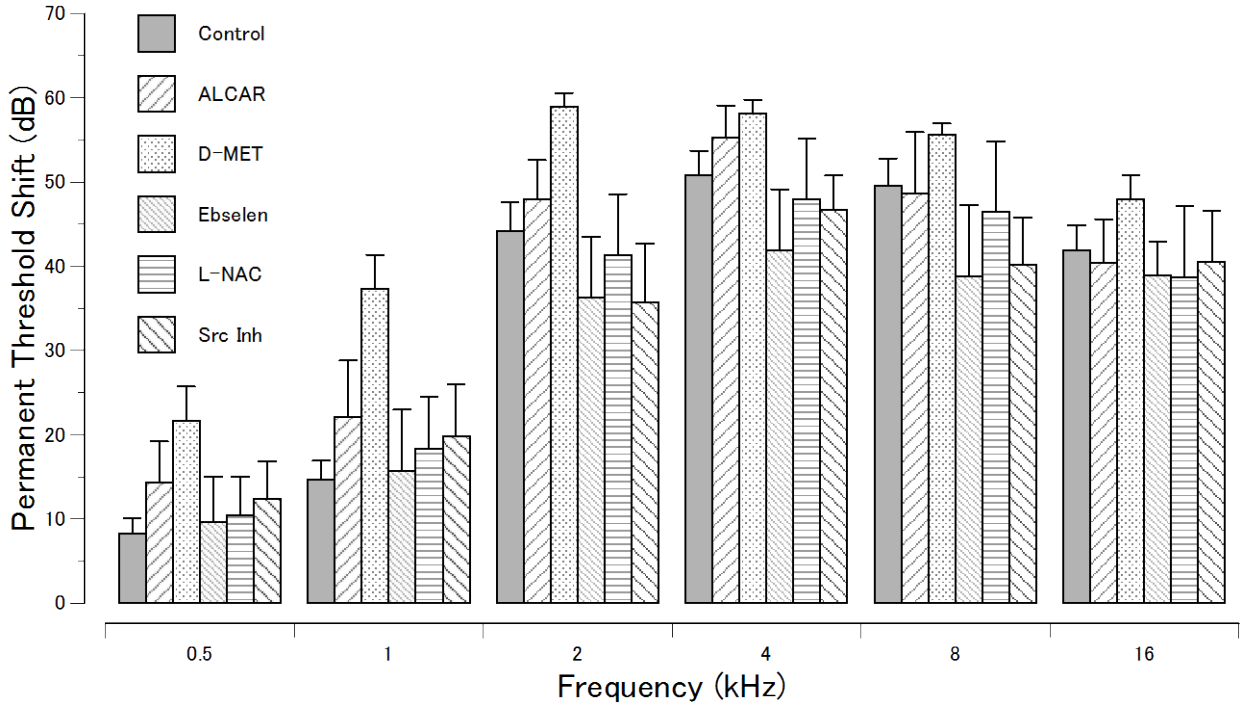


Figure 84. The group mean permanent threshold shift for the control group and the five drug treated groups following exposure for 6 hours to a 4 kHz octave band of noise at 108 dB SPL. T = standard error of the mean.

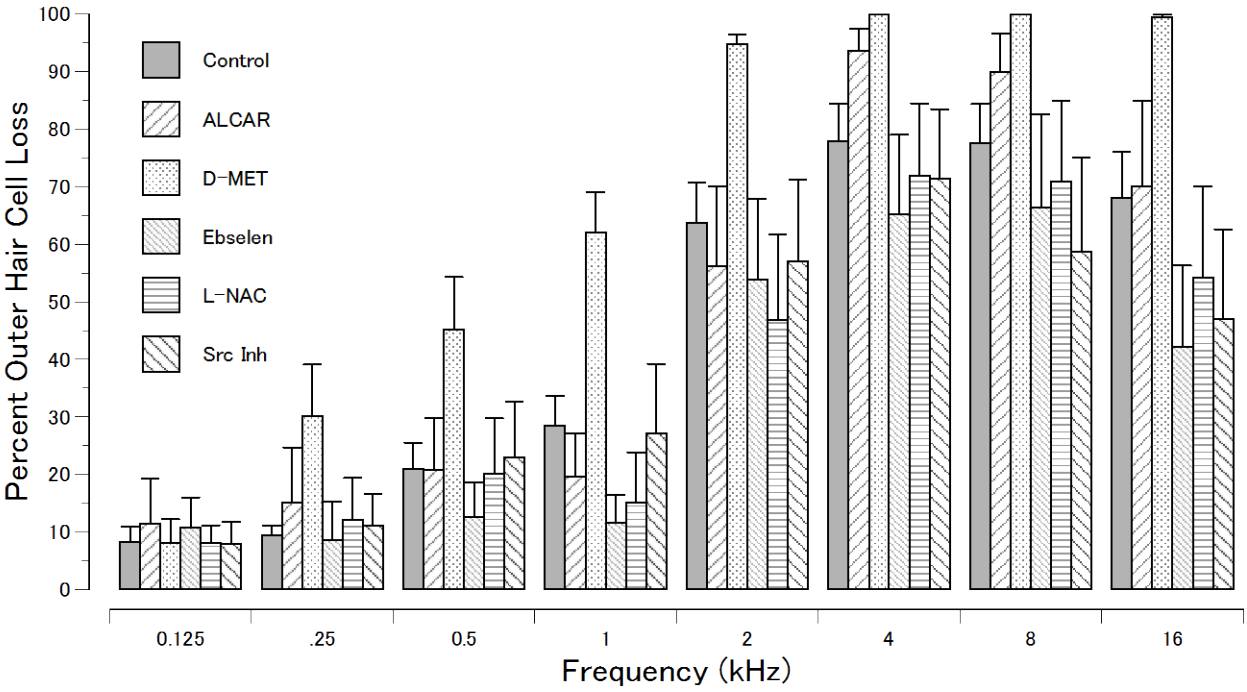


Figure 85. The group mean outer hair cell loss in the indicated octave band length of the basilar membrane for the control group and the five drug treated groups following exposure for 6 hours to a 4 kHz octave band of noise at 108 dB SPL. T = standard error of the mean.

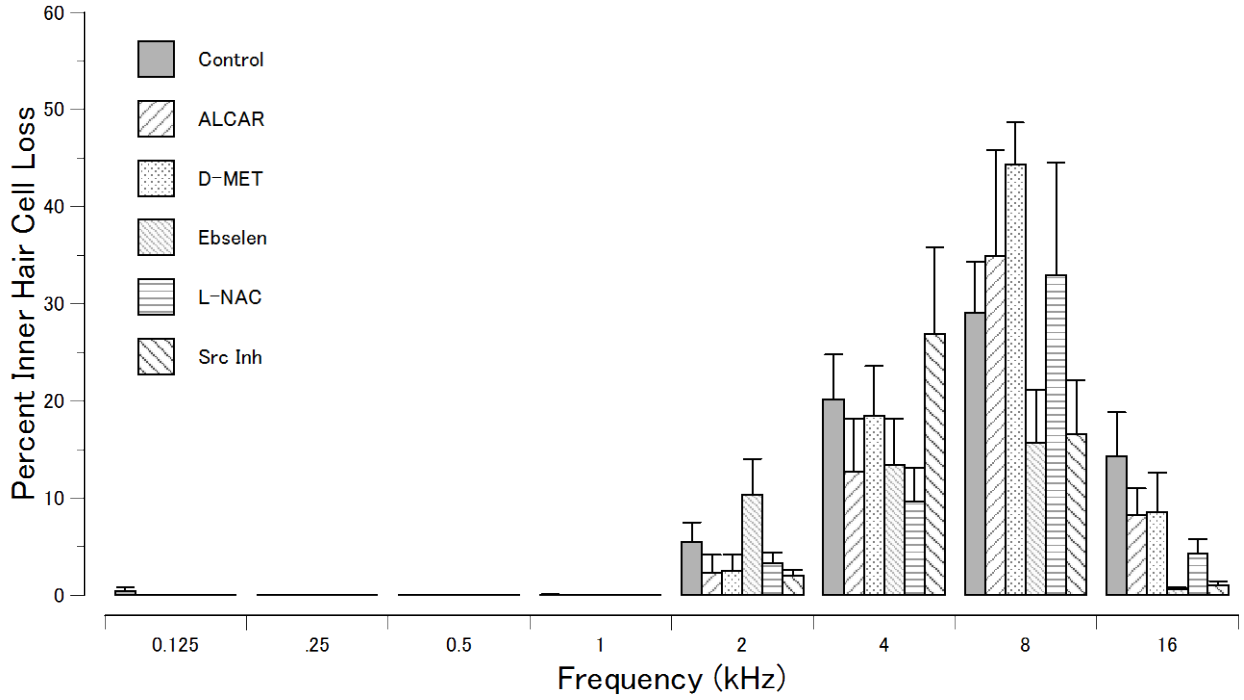


Figure 86. The group mean inner hair cell loss in the indicated octave band length of the basilar membrane for the control group and the five drug treated groups following exposure for 6 hours to a 4 kHz octave band of noise at 108 dB SPL. T = standard error of the mean.

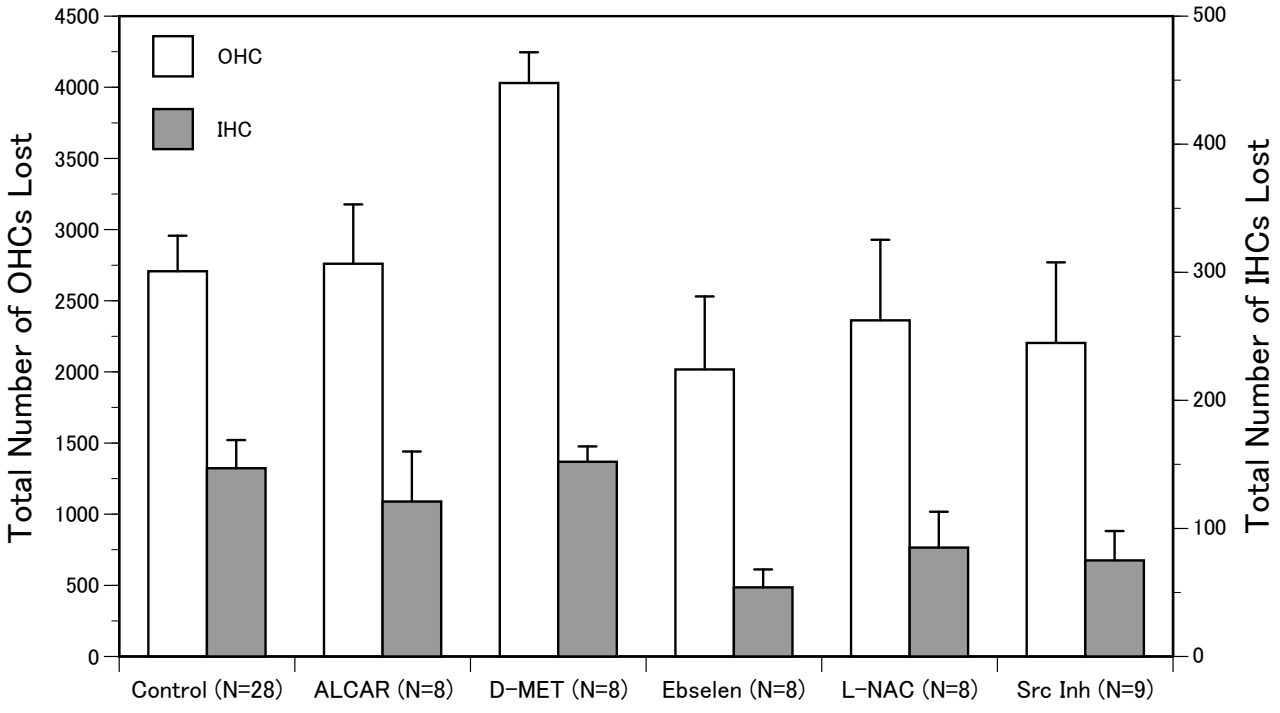


Figure 87. The group mean total number of missing outer hair cells (OHC) and inner hair cells (IHC) in the control and five drug treated groups that were exposed to the 4 kHz octave band of noise at 108 dB for 6 hours. T = standard error of the mean.

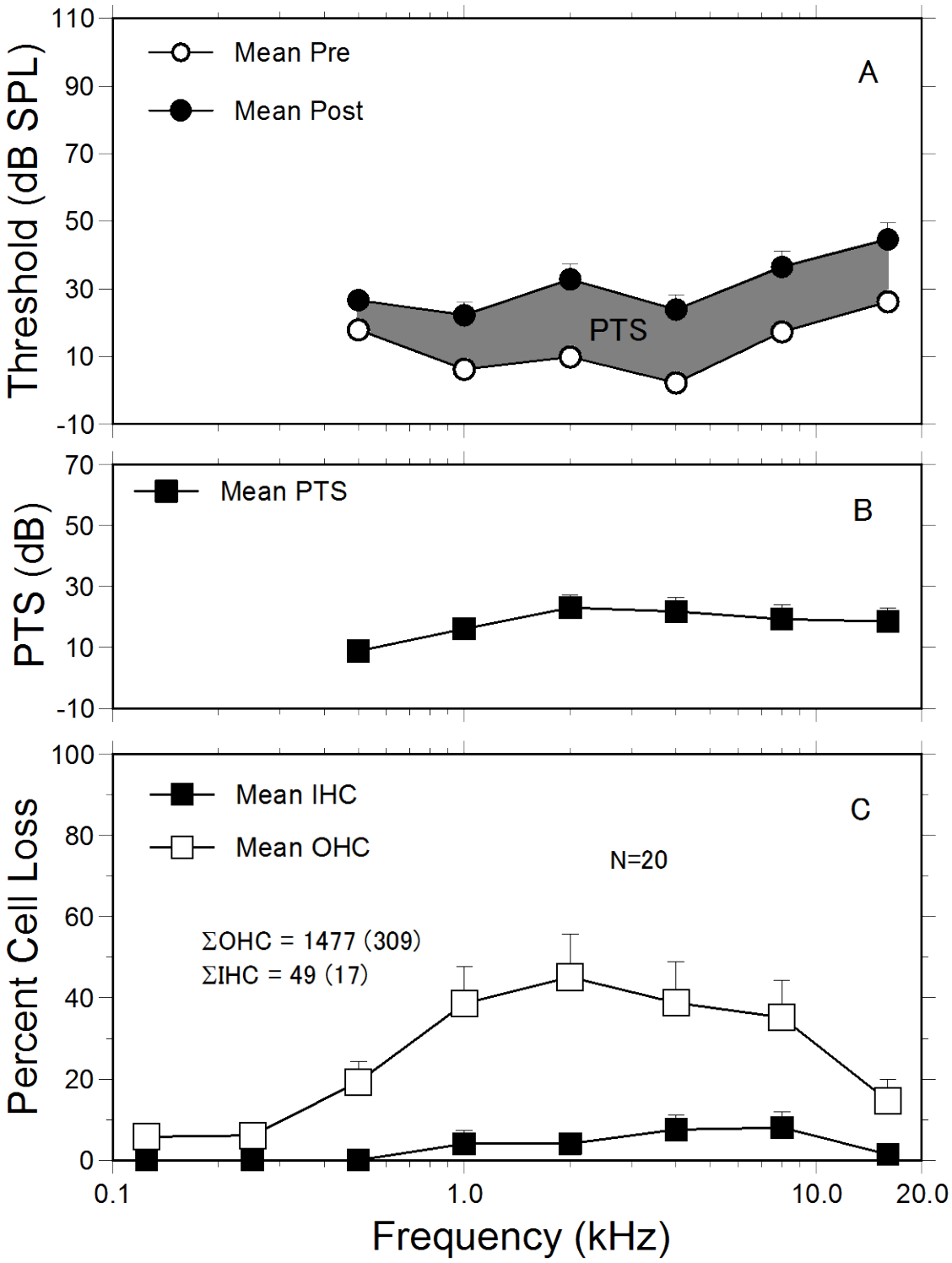


Figure 88. Phase III: The first noise only control group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. (T = standard error).

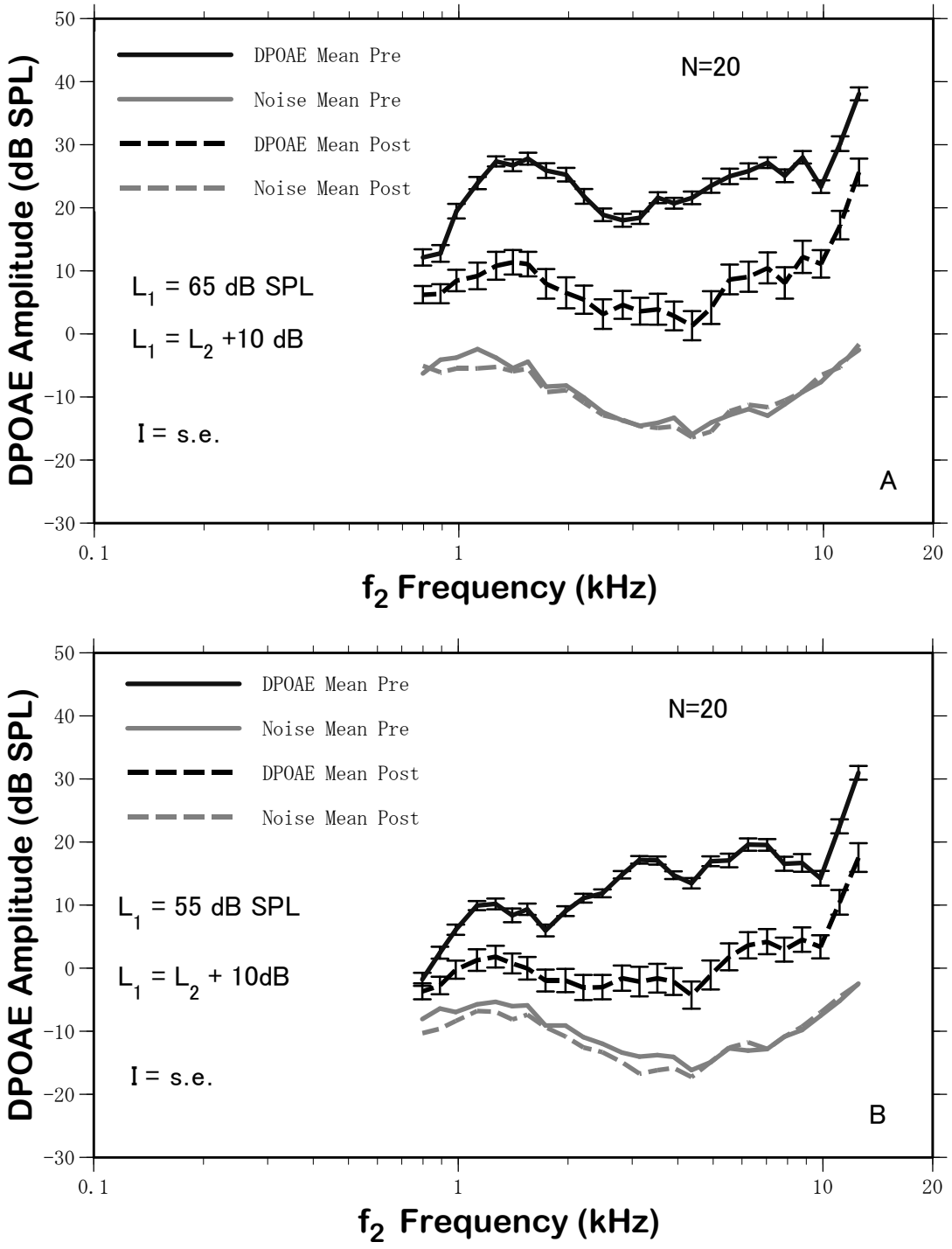


Figure 89. Phase III: The first noise only control group (N=20) mean pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. (I = s.e.)

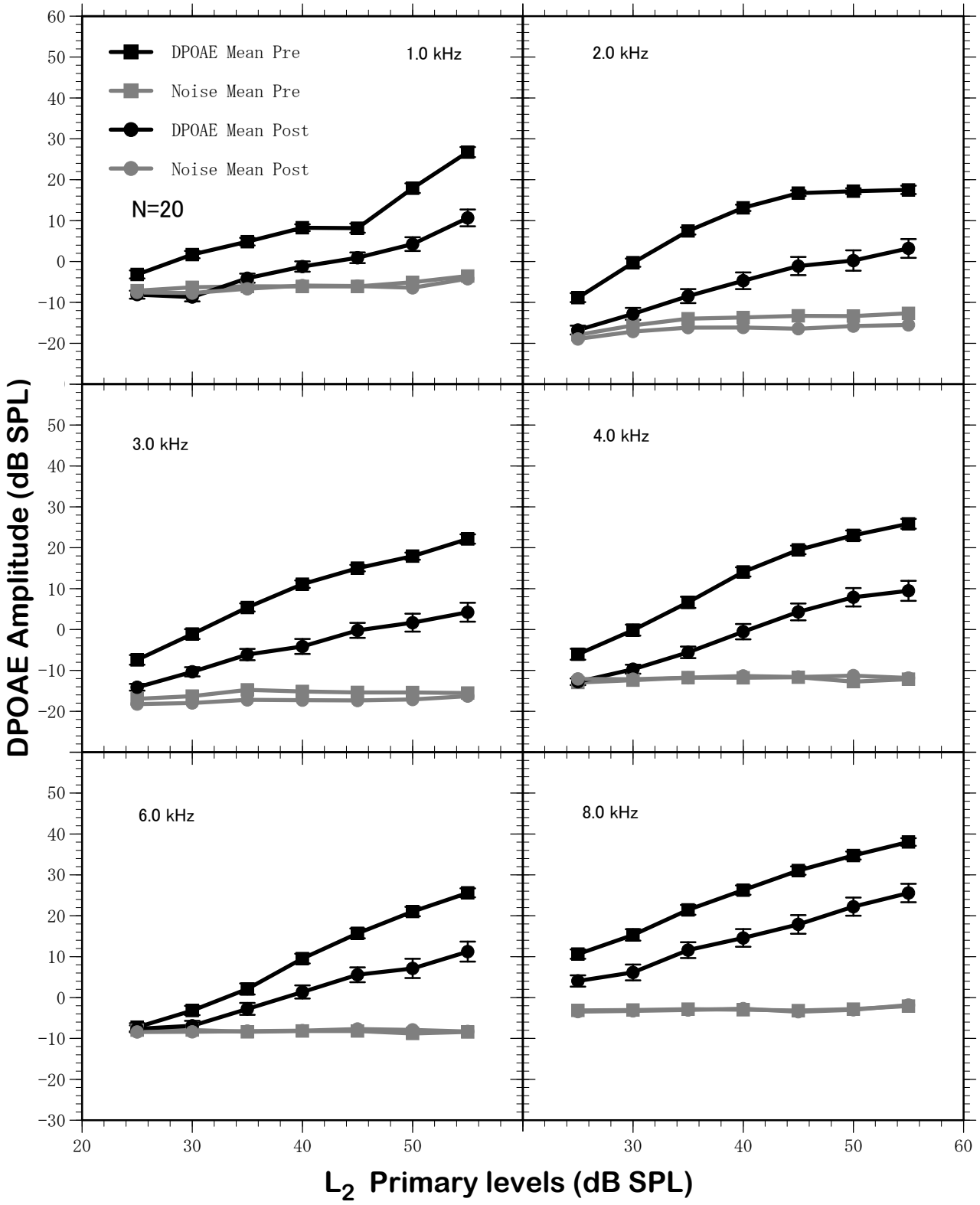


Figure 90. Phase III: The first noise only control group (N=20) mean pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. (I = s.e.)

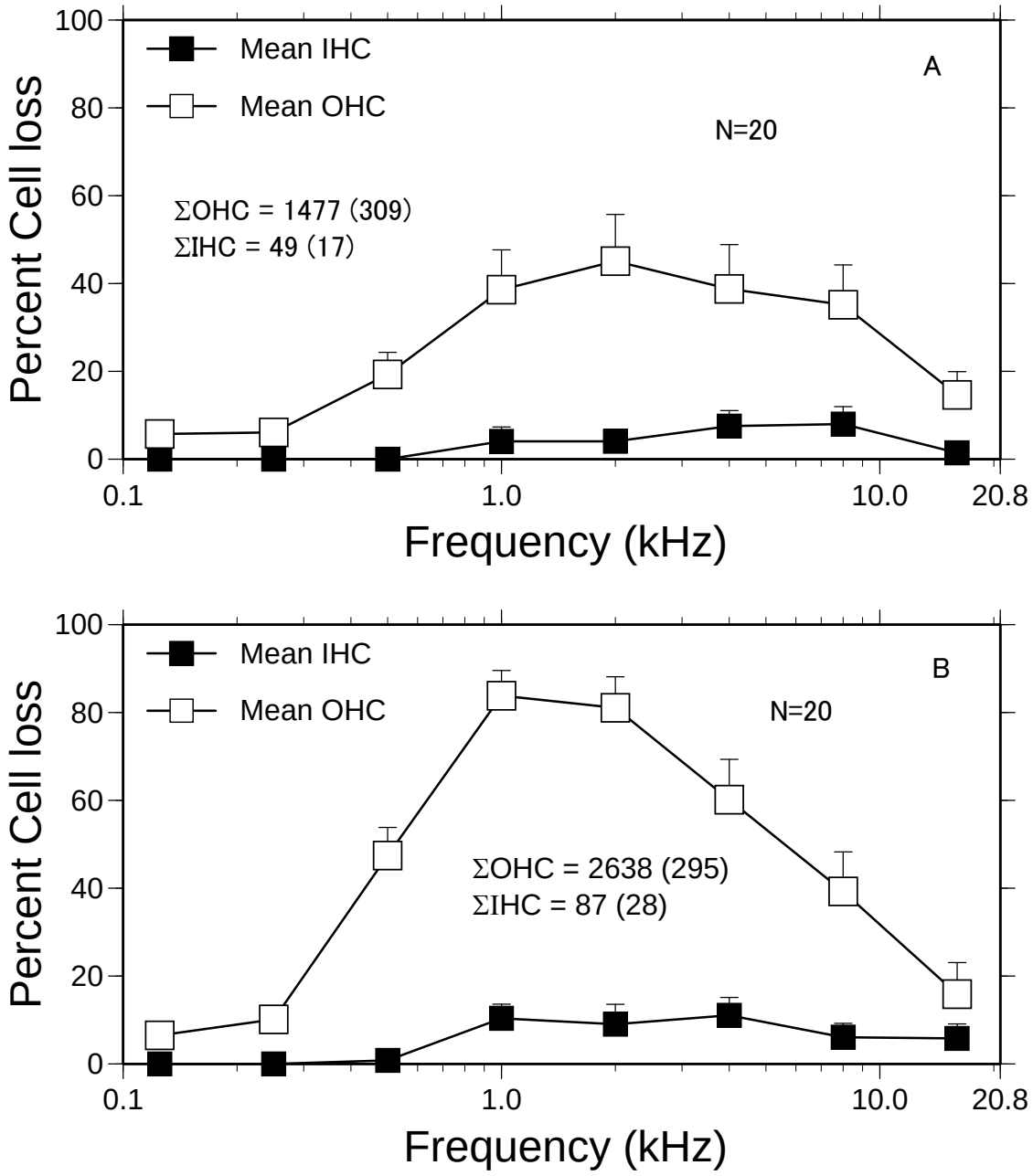


Figure 91. A comparison of the group (N=20) mean cochleogram from the first noise (blast wave) only control group and that of the Phase I lesion calibration (see Fig. 11 and 13) group. Exposure: 158 dB peak SPL at the subject's ear, 10X (11 psi charge pressure). (T = standard error).

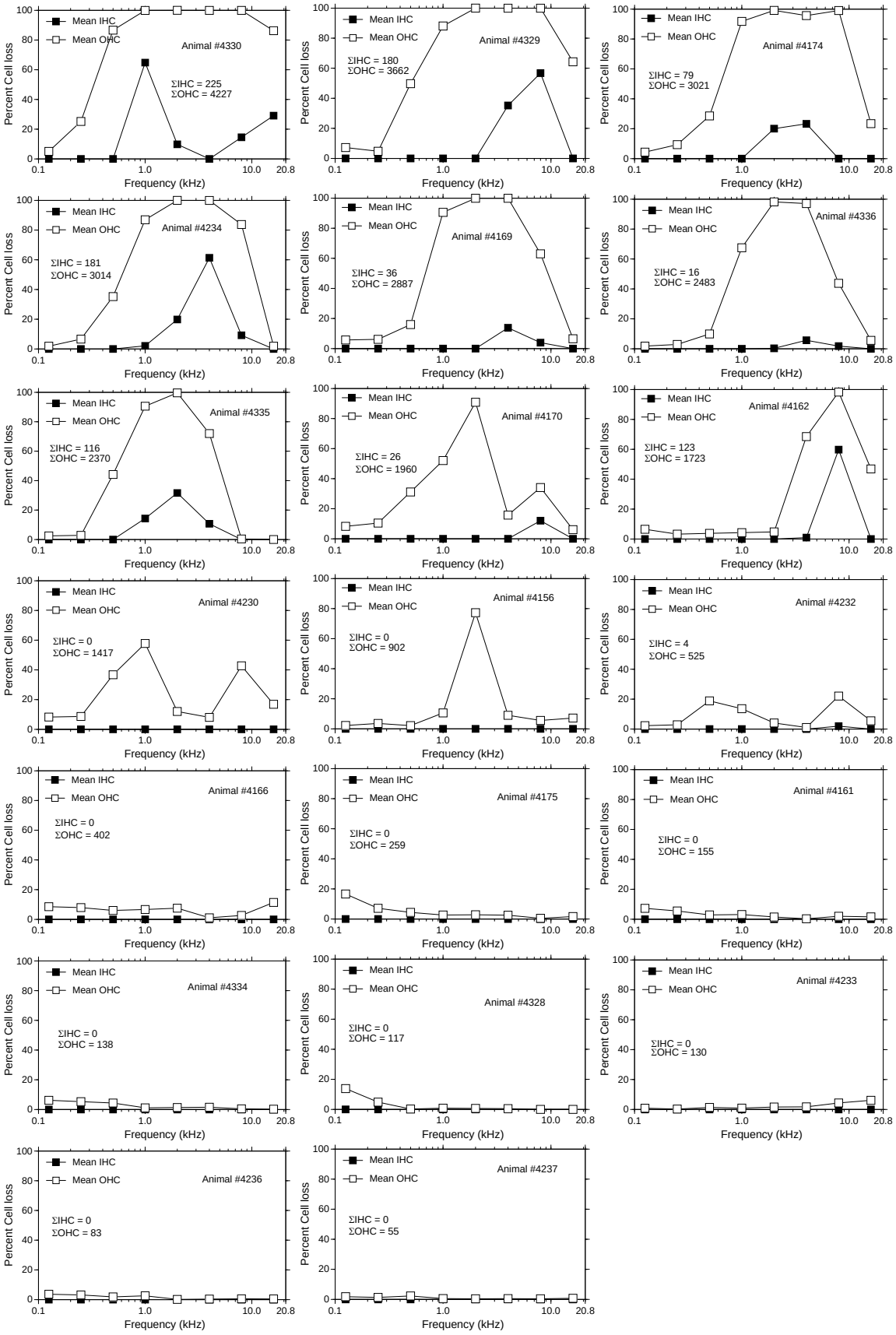


Figure 92. Phase III: Individual cochleograms of animals in the first noise only control group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min.

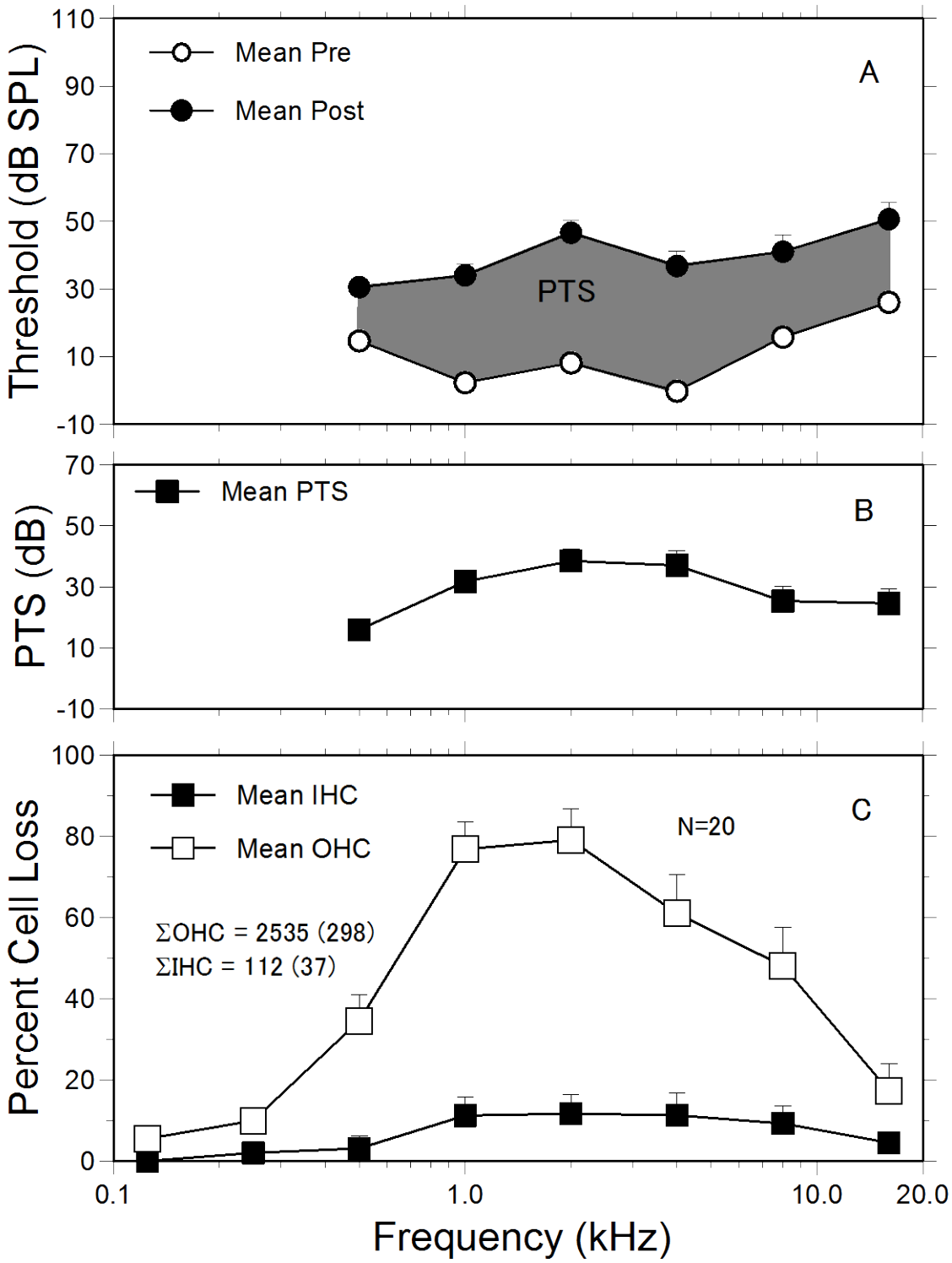


Figure 93. Phase III: The second noise only control group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. (T = standard error).

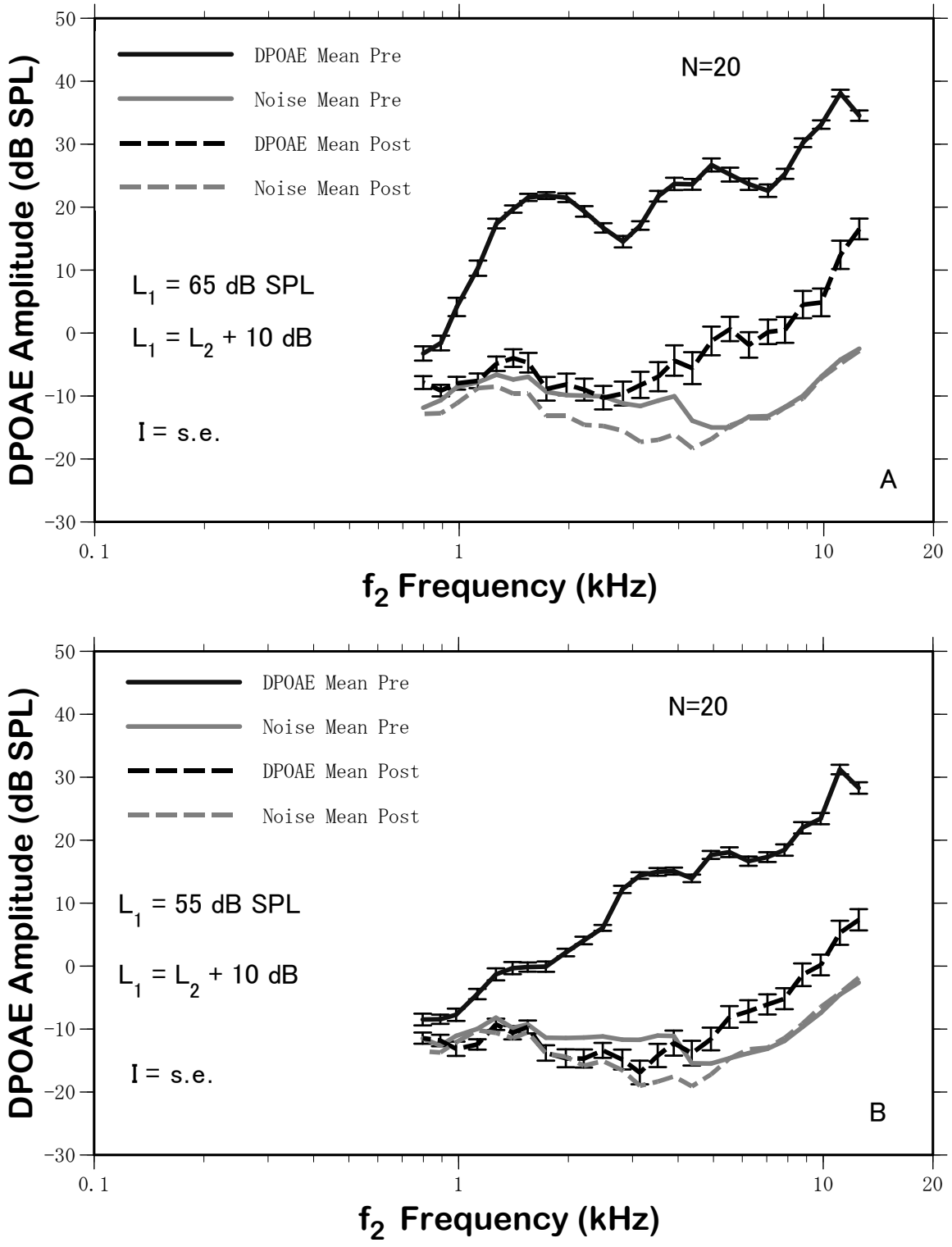


Figure 94. Phase III: The second noise only group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. (I = s.e.)

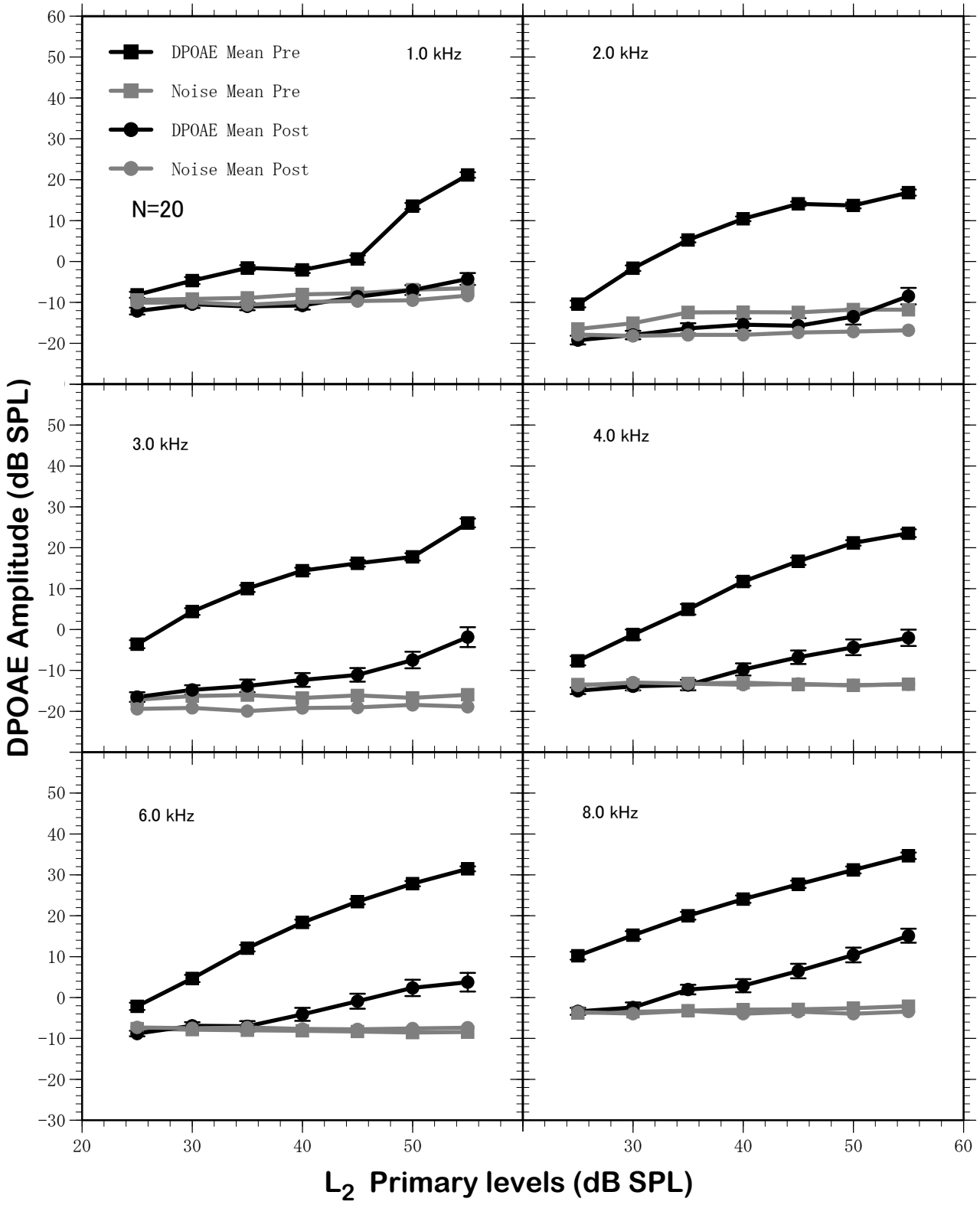


Figure 95. Phase III: The second noise only group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. (I = s.e.)

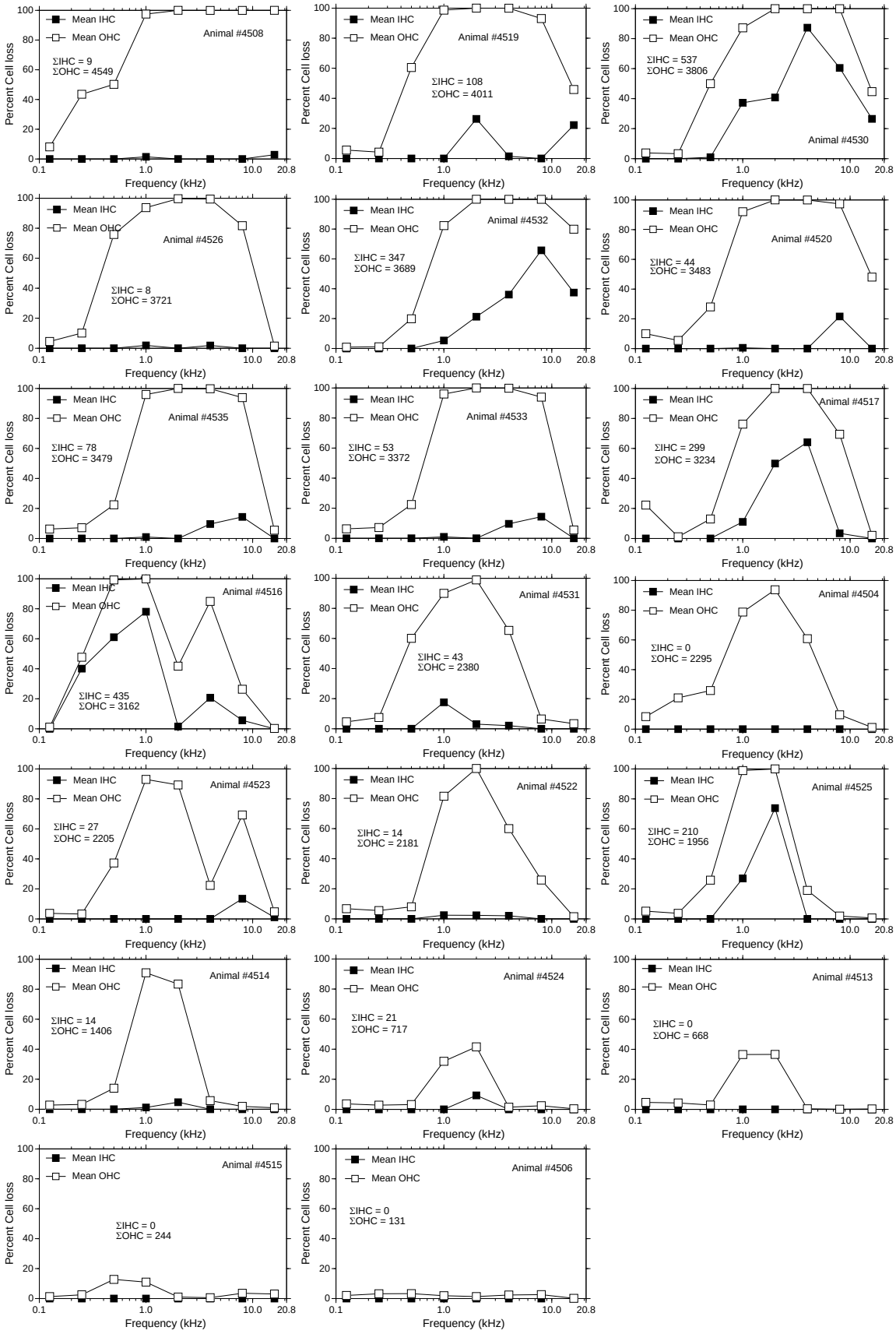


Figure 96. Phase III: Individual cochleograms of animals in the second noise only control group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min.

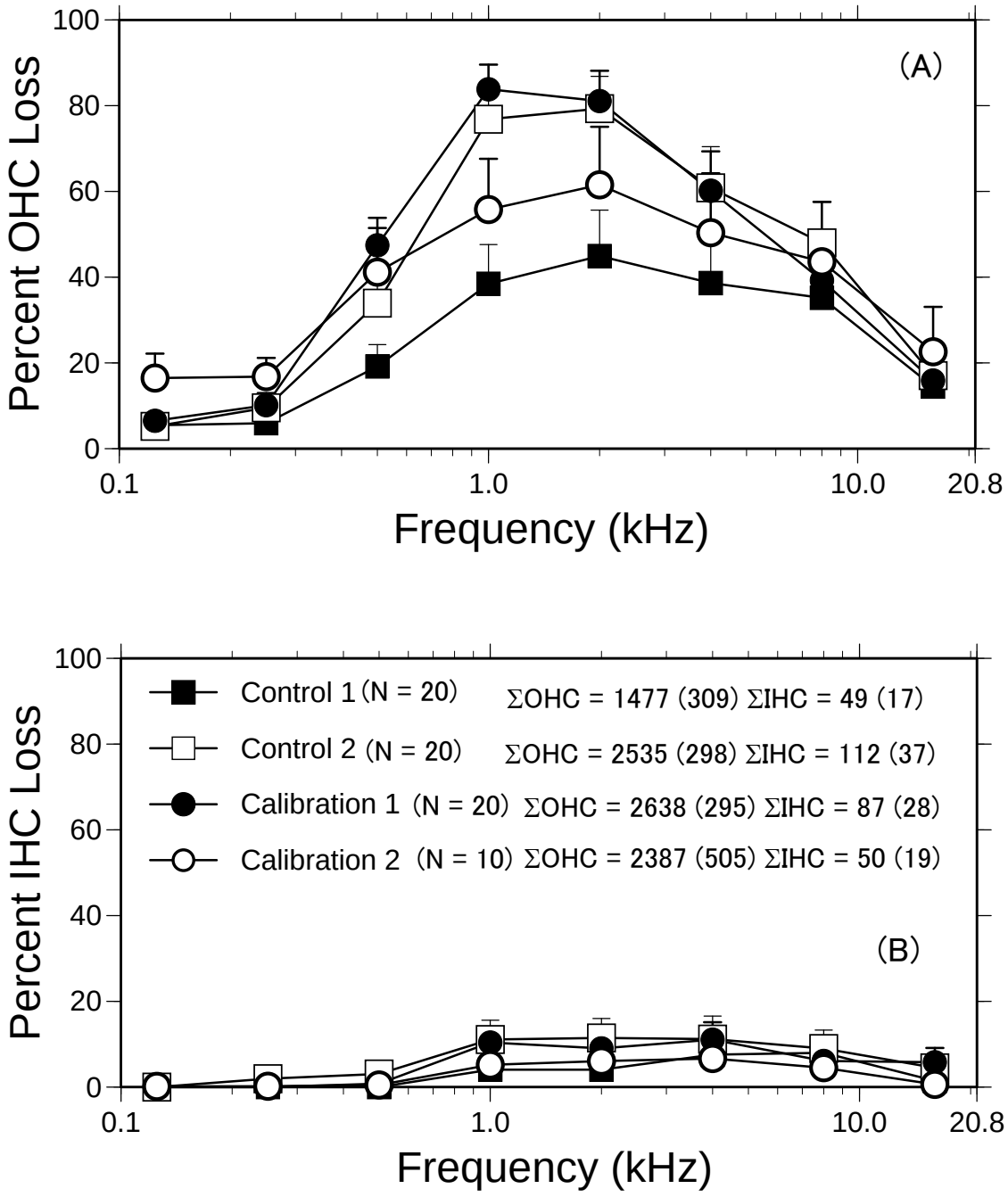


Figure 97. The group mean (A) %OHC and (B) %IHC loss for the two noise only control groups and the two shock tube calibration groups. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

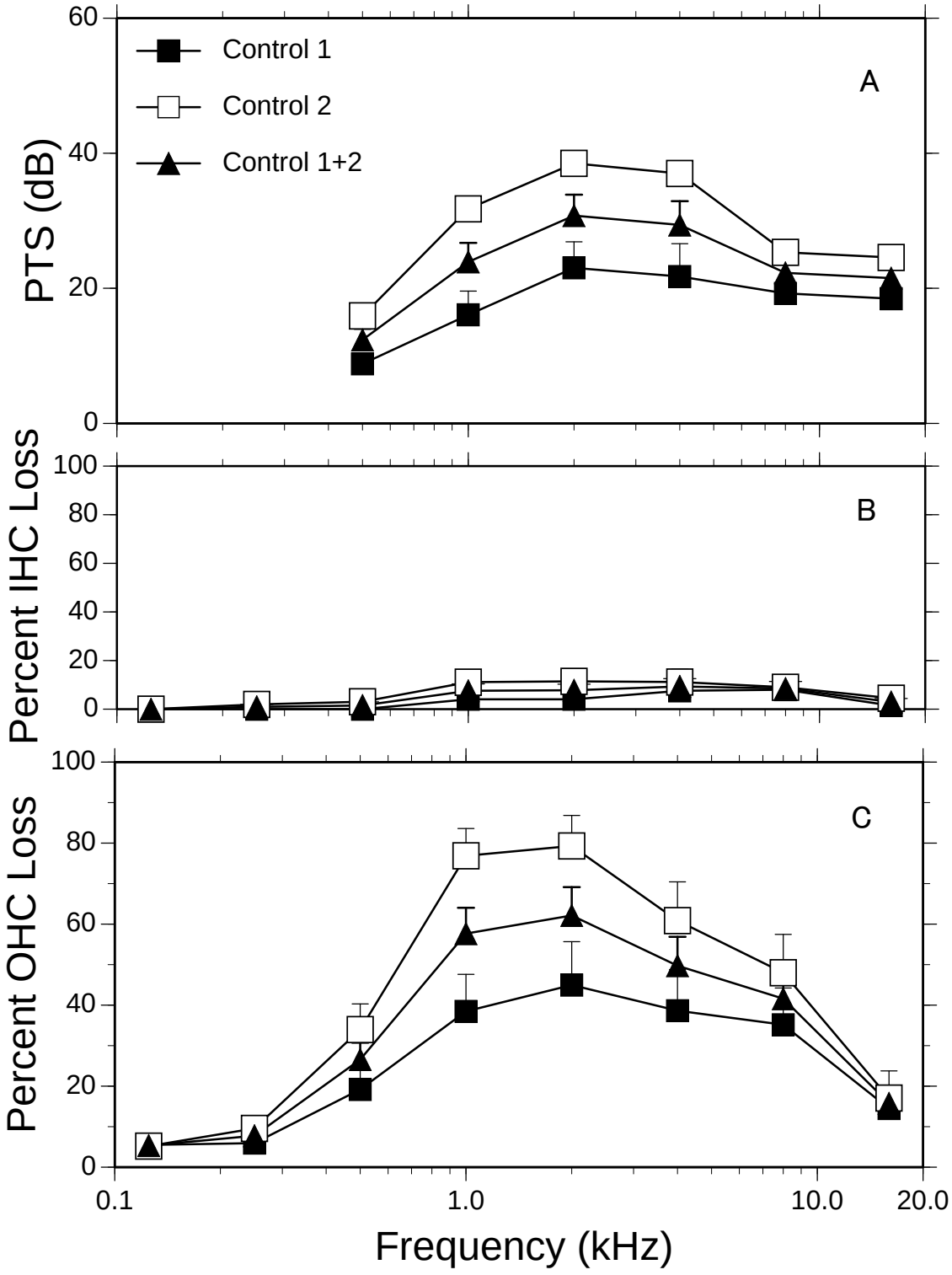


Figure 98. The group mean PTS, %OHC and %IHC loss for control groups #1 and #2, also shown is the mean of the data from control groups #1 and #2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi) within less than 2 min. (T = standard error).

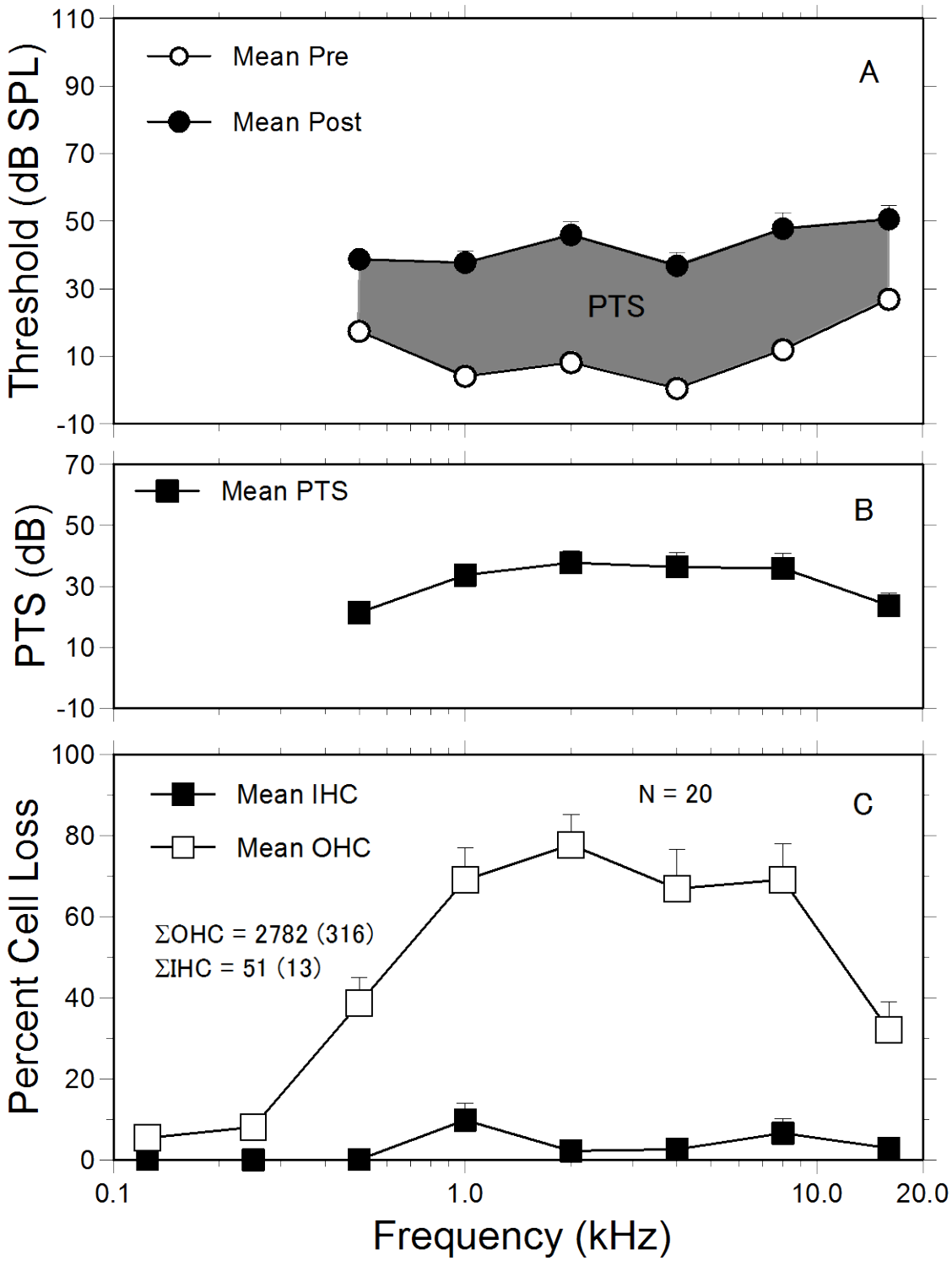


Figure 99. Phase III: L-NAC treated group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

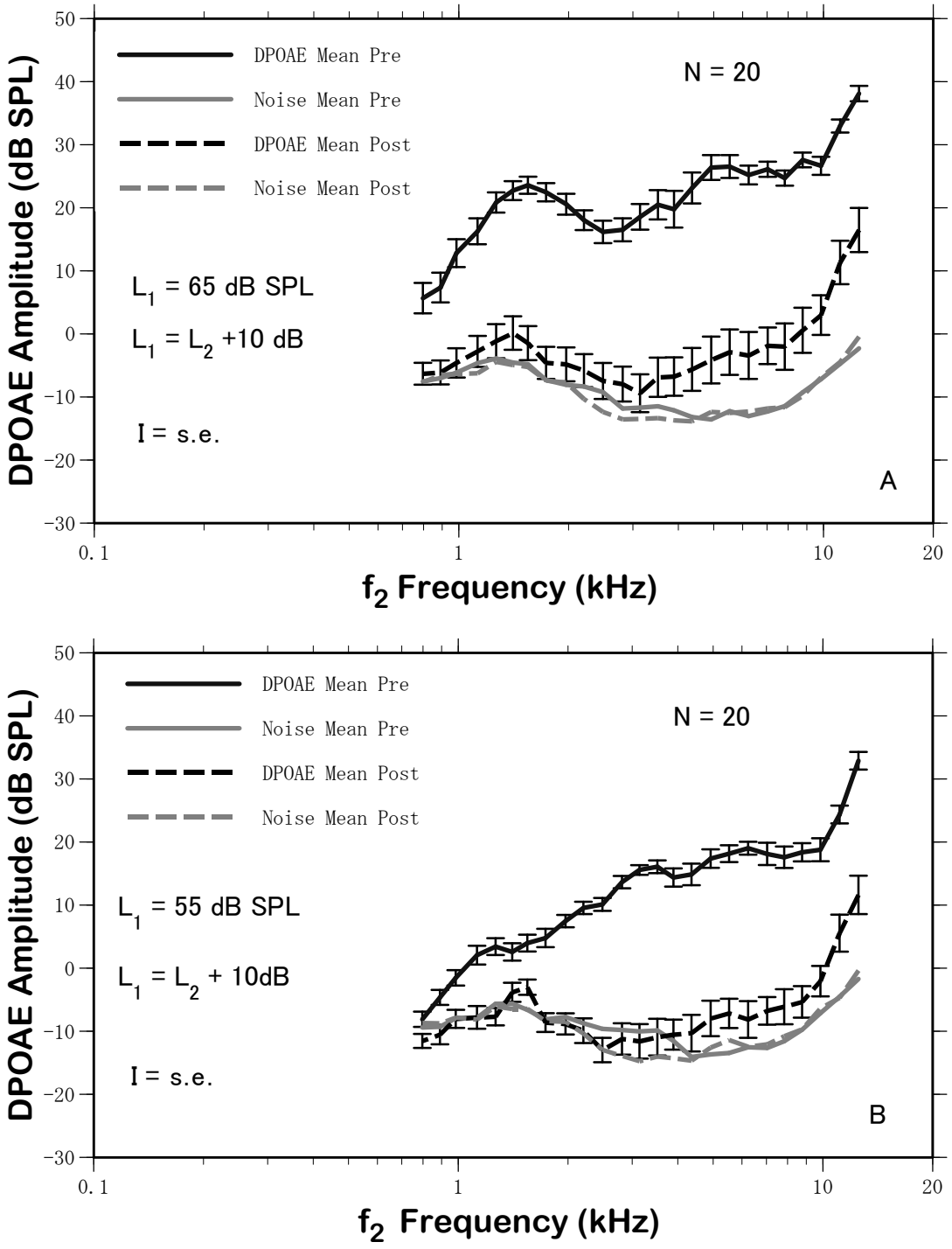


Figure 100. Phase III: L-NAC treated group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

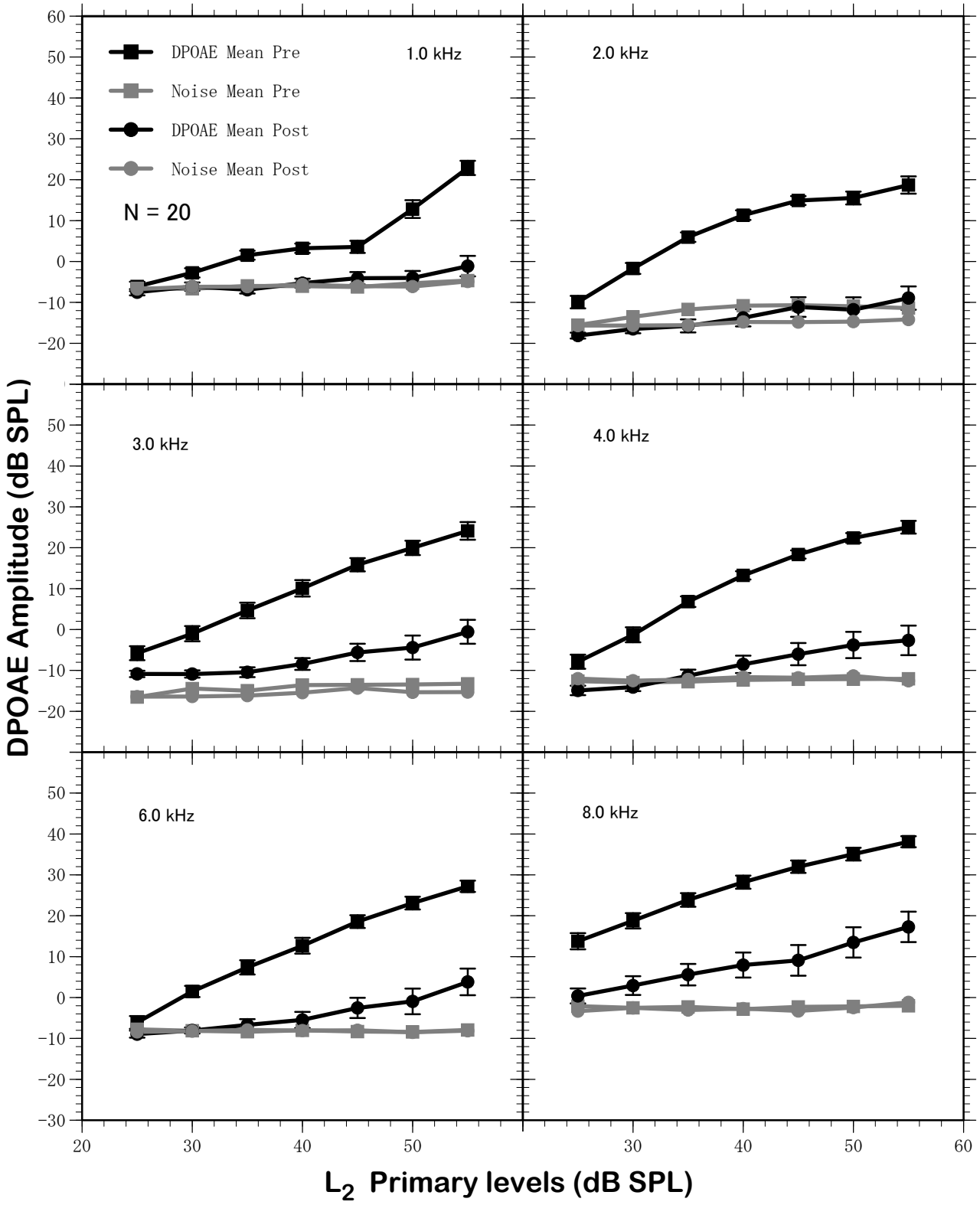


Figure 101. Phase III: L-NAC treated group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

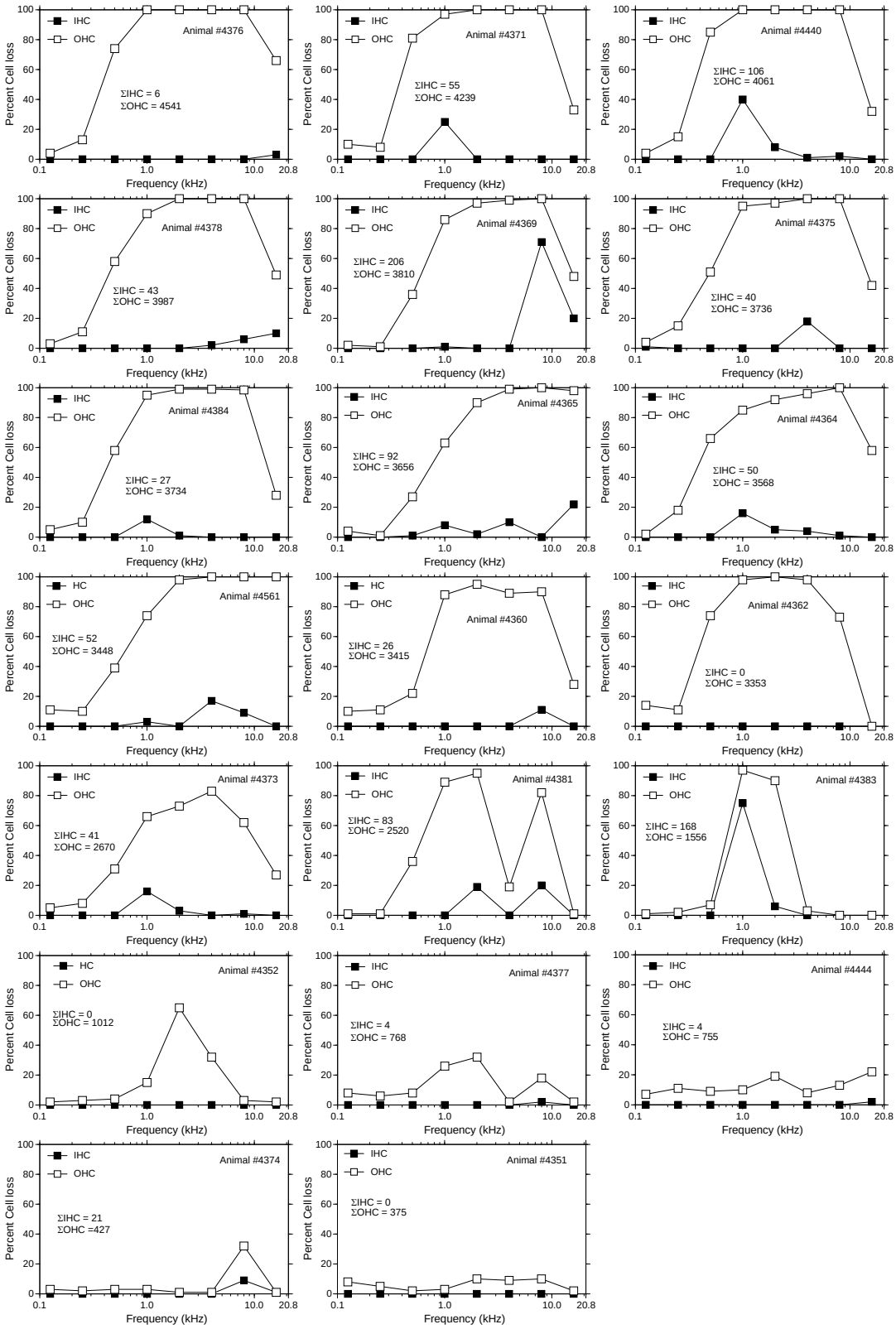


Figure 102. Phase III: Individual cochleograms for the group (N=20) treated with L-NAC in the rescue mode and exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi).

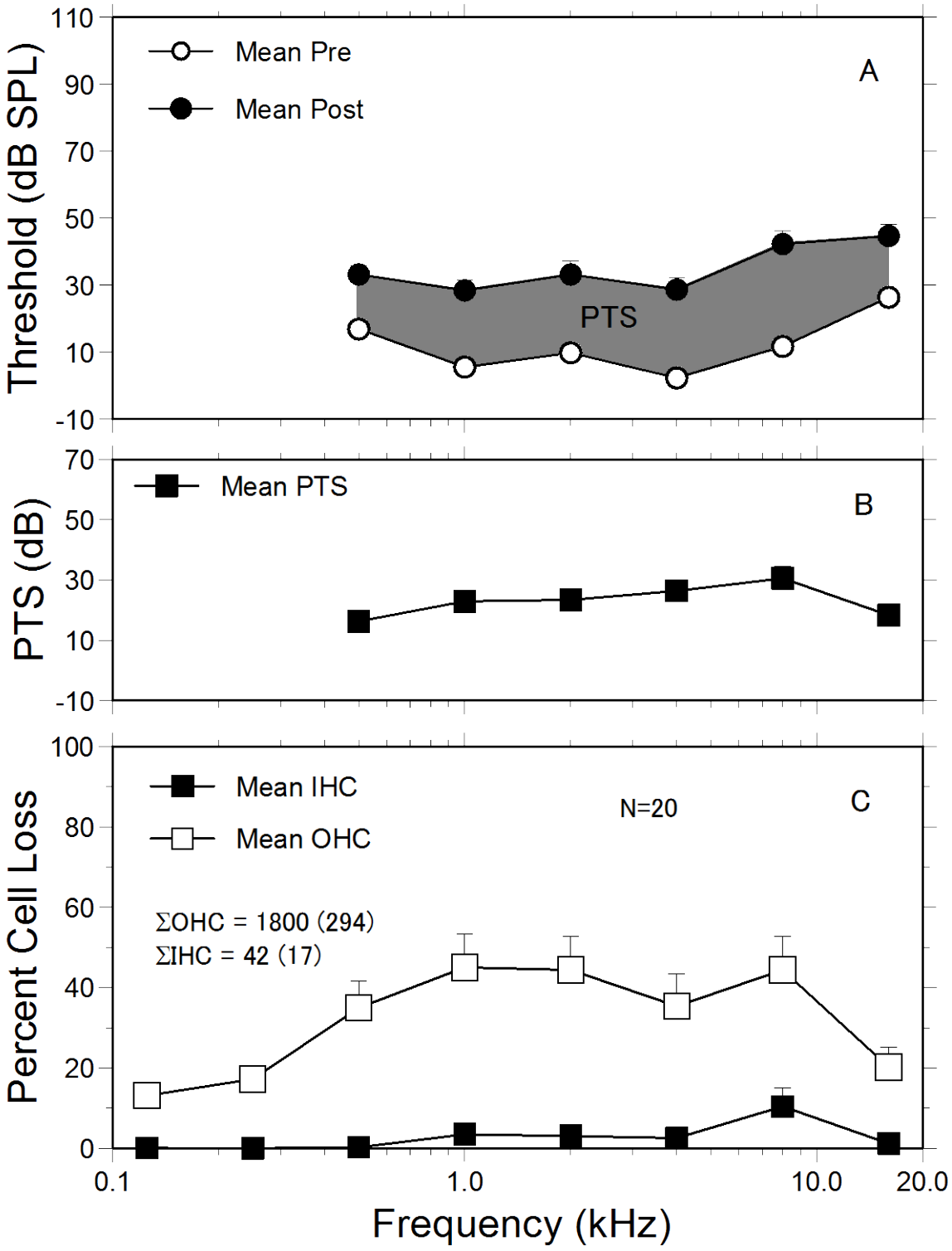


Figure 103. Phase III: D-MET treated group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

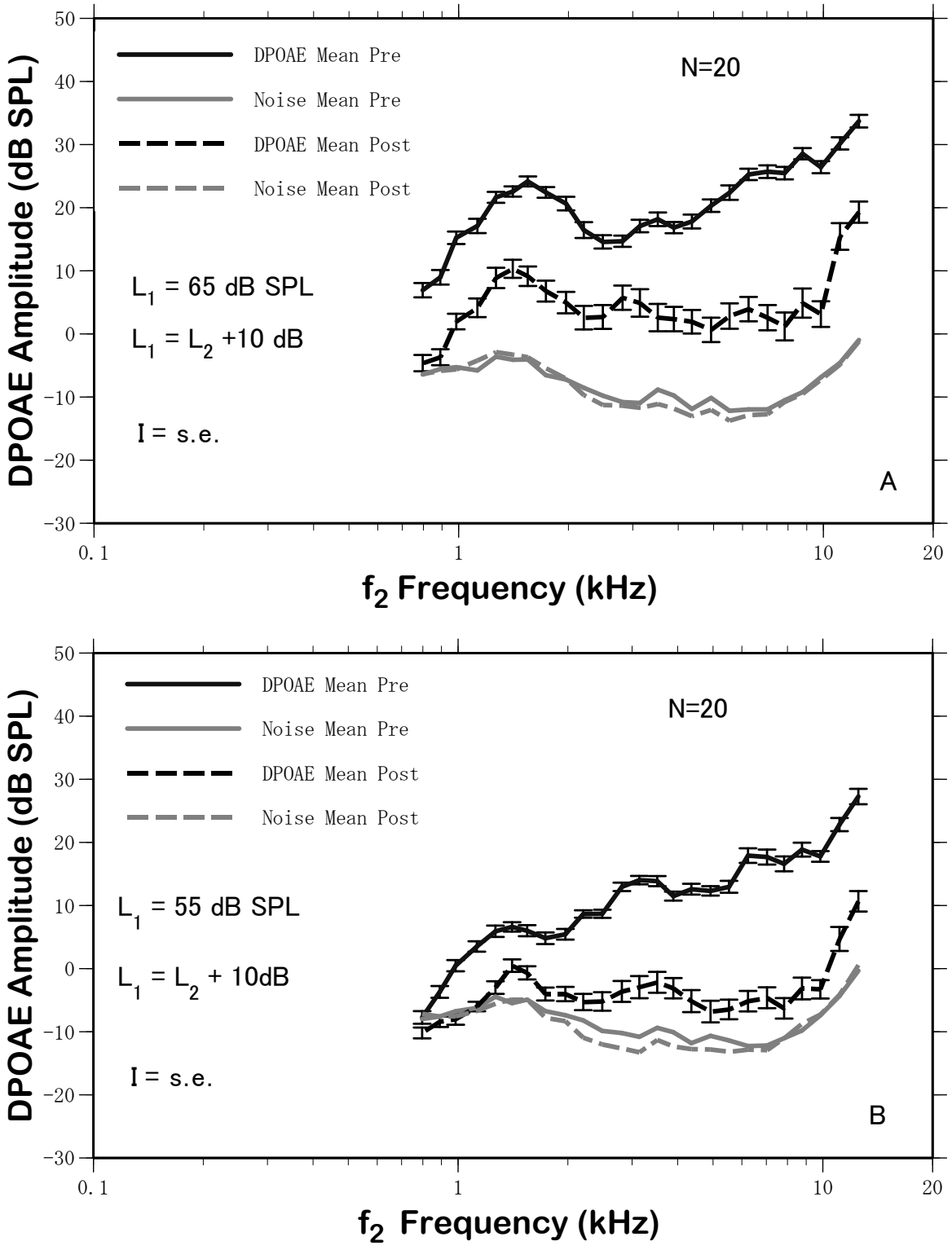


Figure 104. Phase III: D-MET treated group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

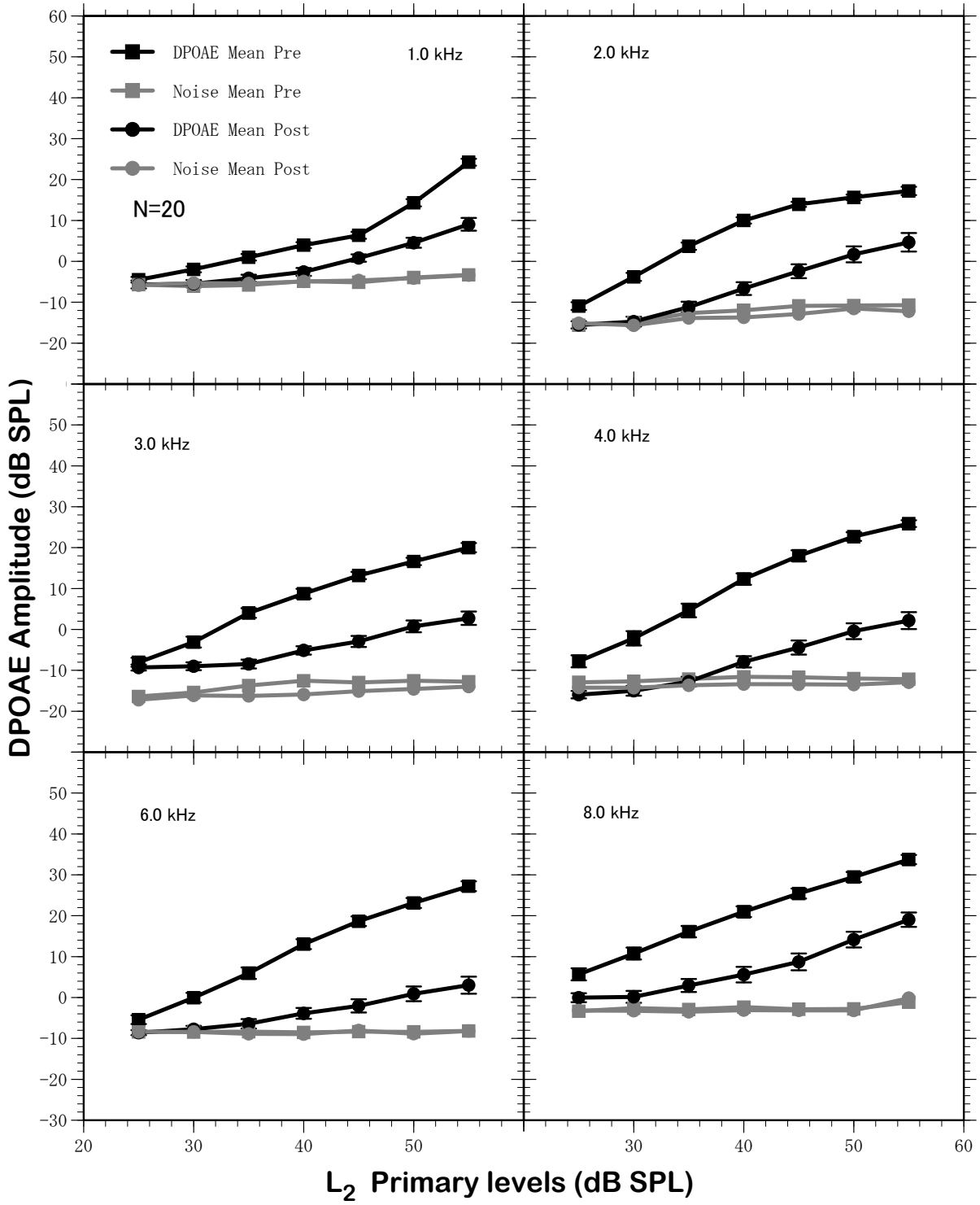


Figure 105. Phase III: D-MET treated group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

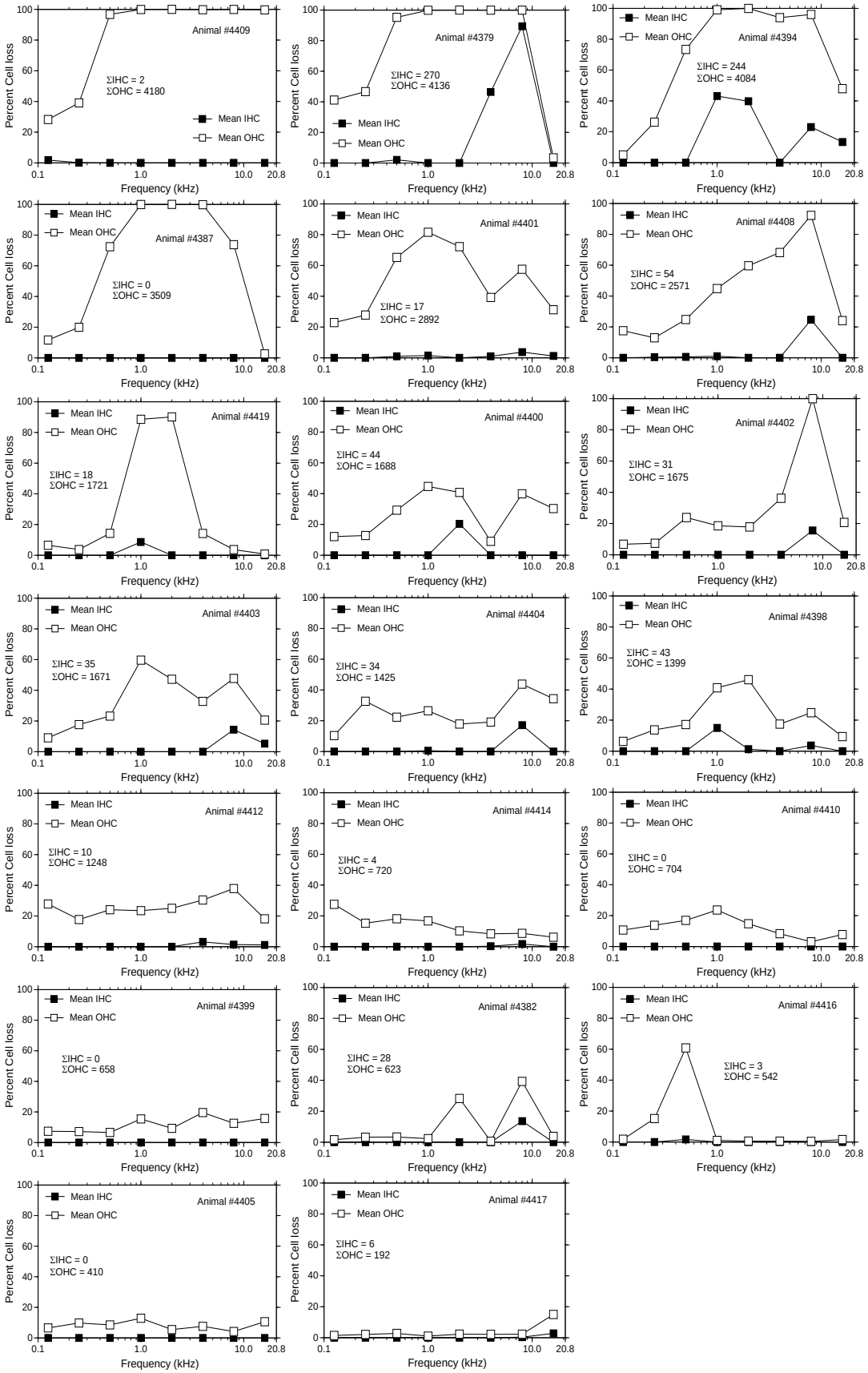


Figure 106. Phase III: Individual cochleograms for the group (N=20) treated with D-MET in the rescue mode and exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi).

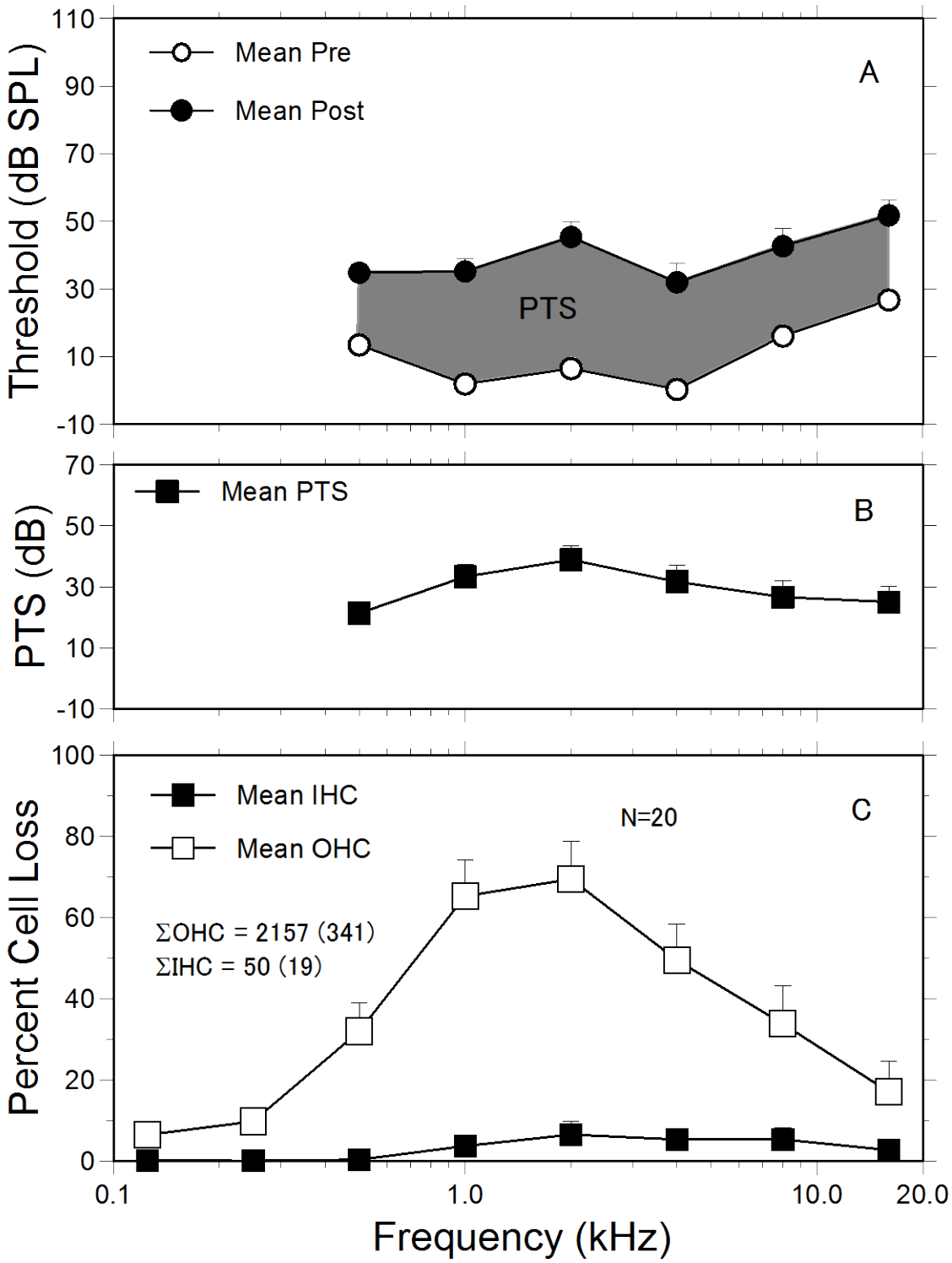


Figure 107. Phase III: ALCAR treated group (N=20) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

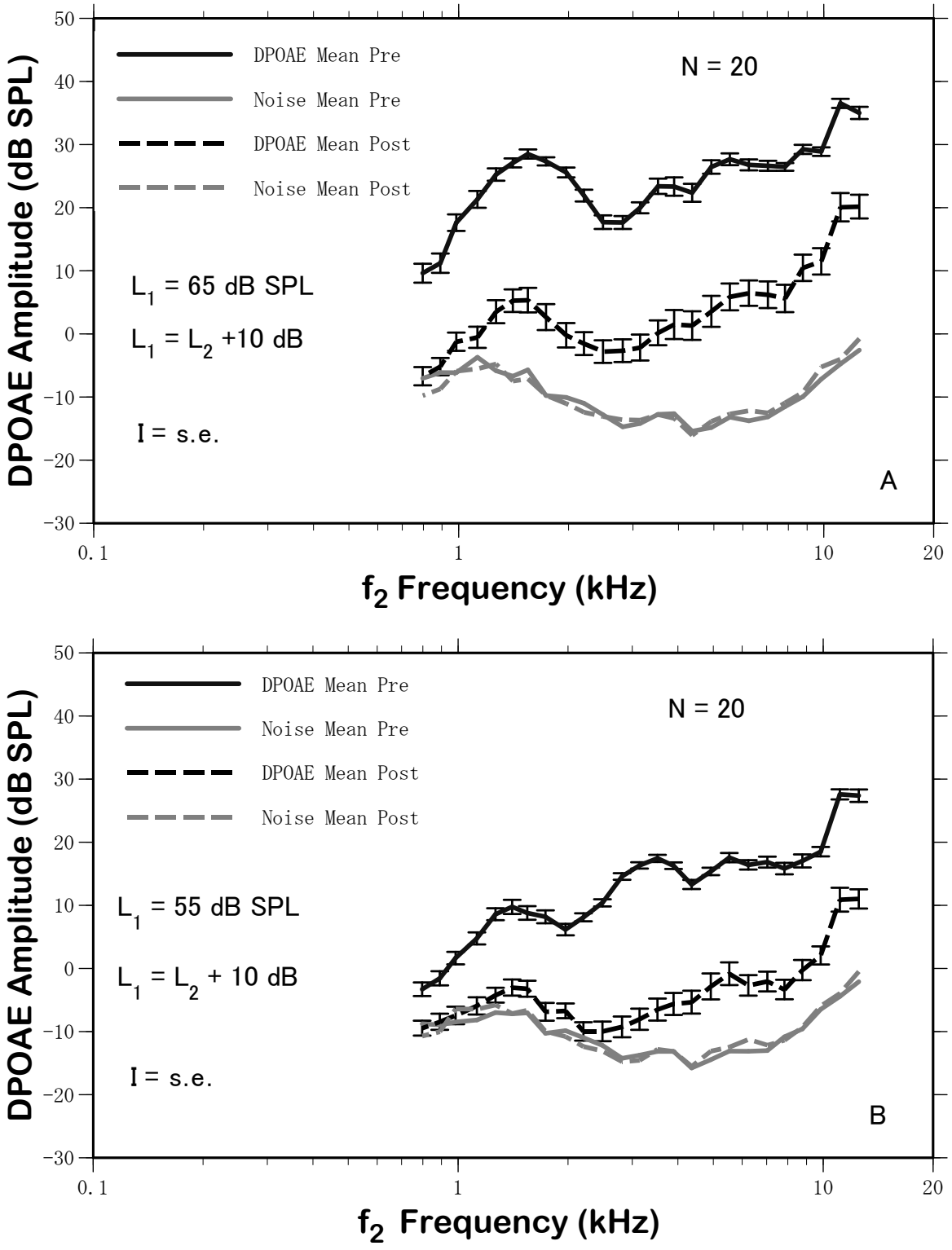


Figure 108. Phase III: ALCAR treated group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

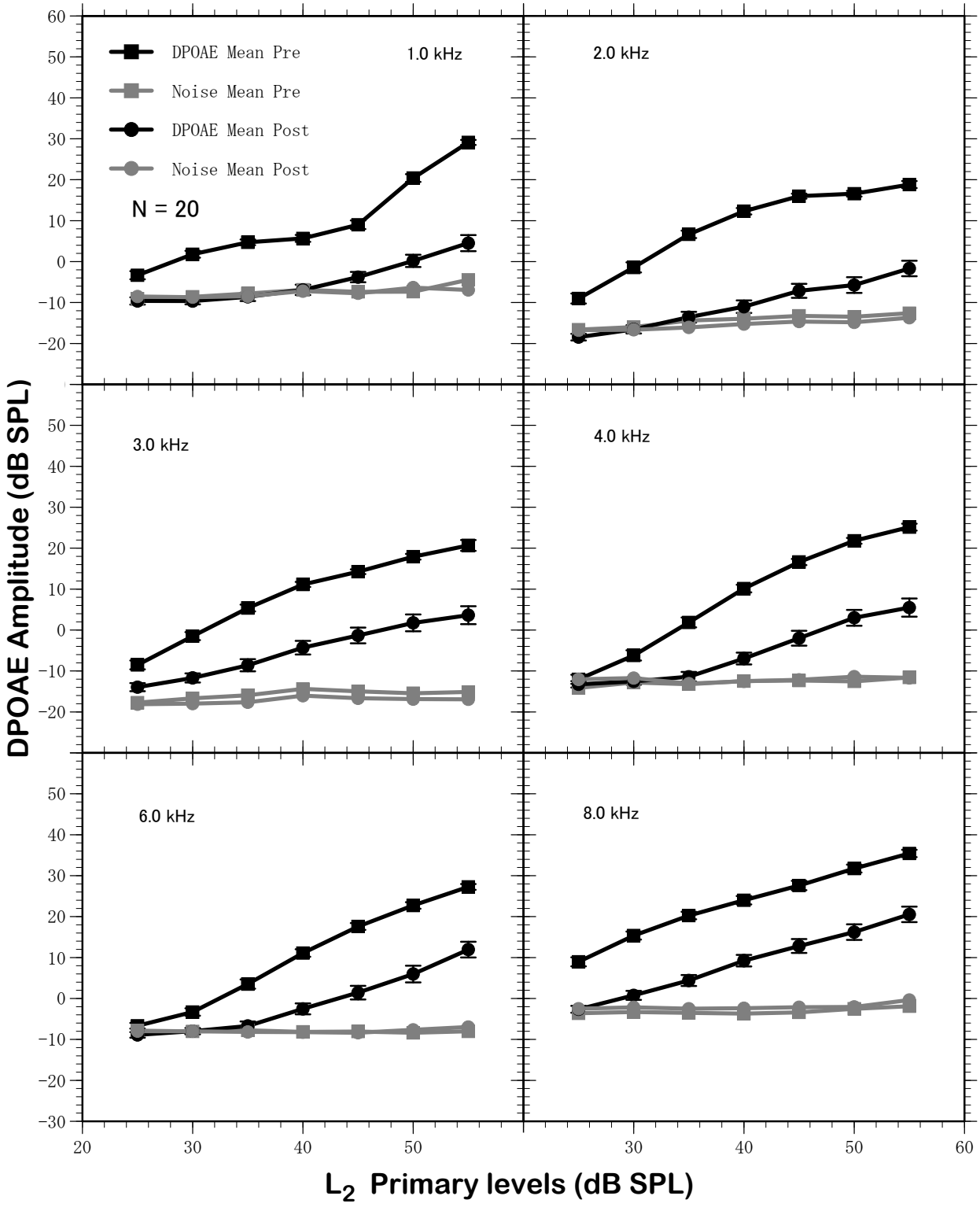


Figure 109. Phase III: ALCAR treated group mean (N=20) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

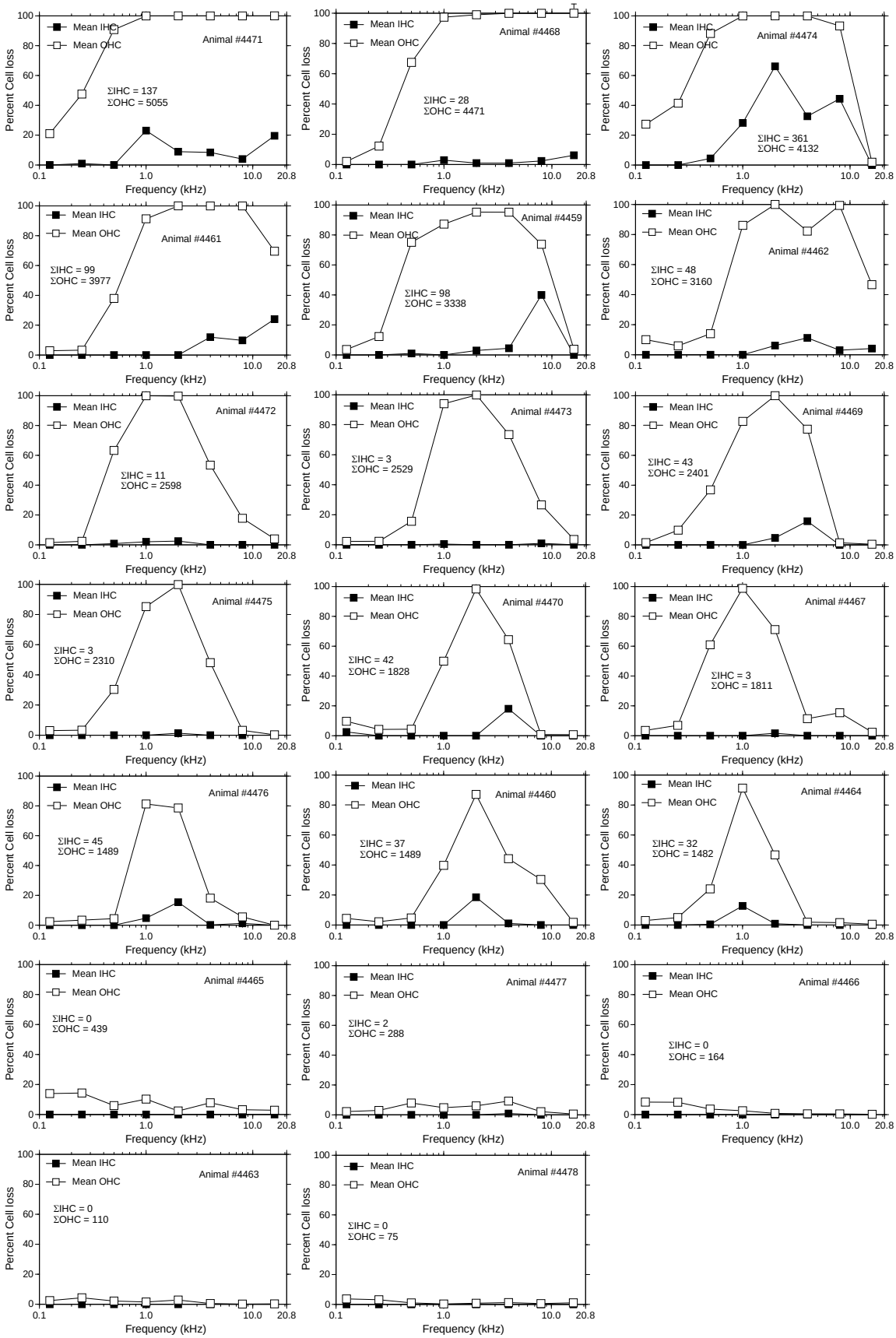


Figure 110. Phase III: Individual cochleograms for the group (N=20) treated with ALCAR in the rescue mode and exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi).

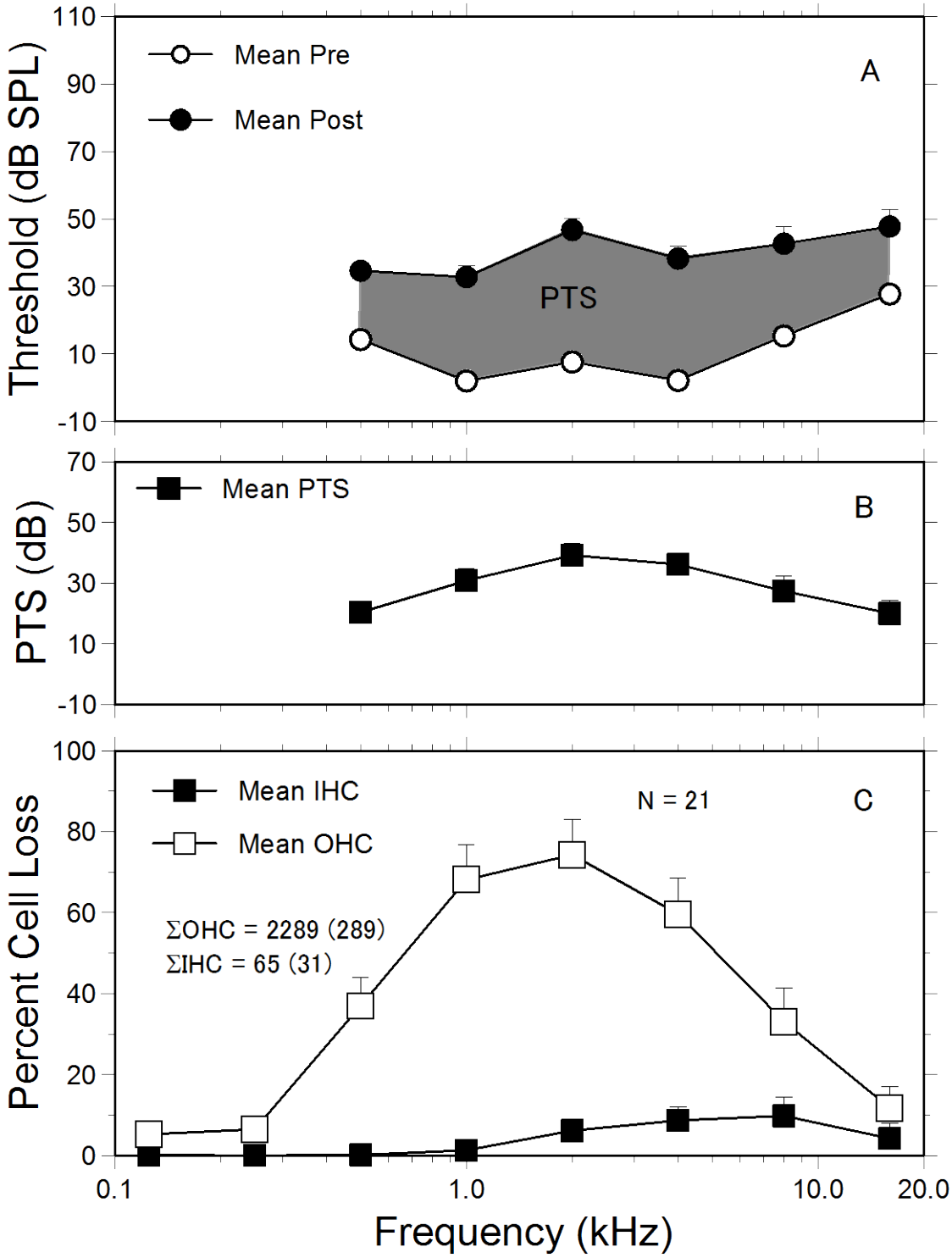


Figure 111. Phase III: Src Inh treated group (N=21) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer hair cell (%IHC, %OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

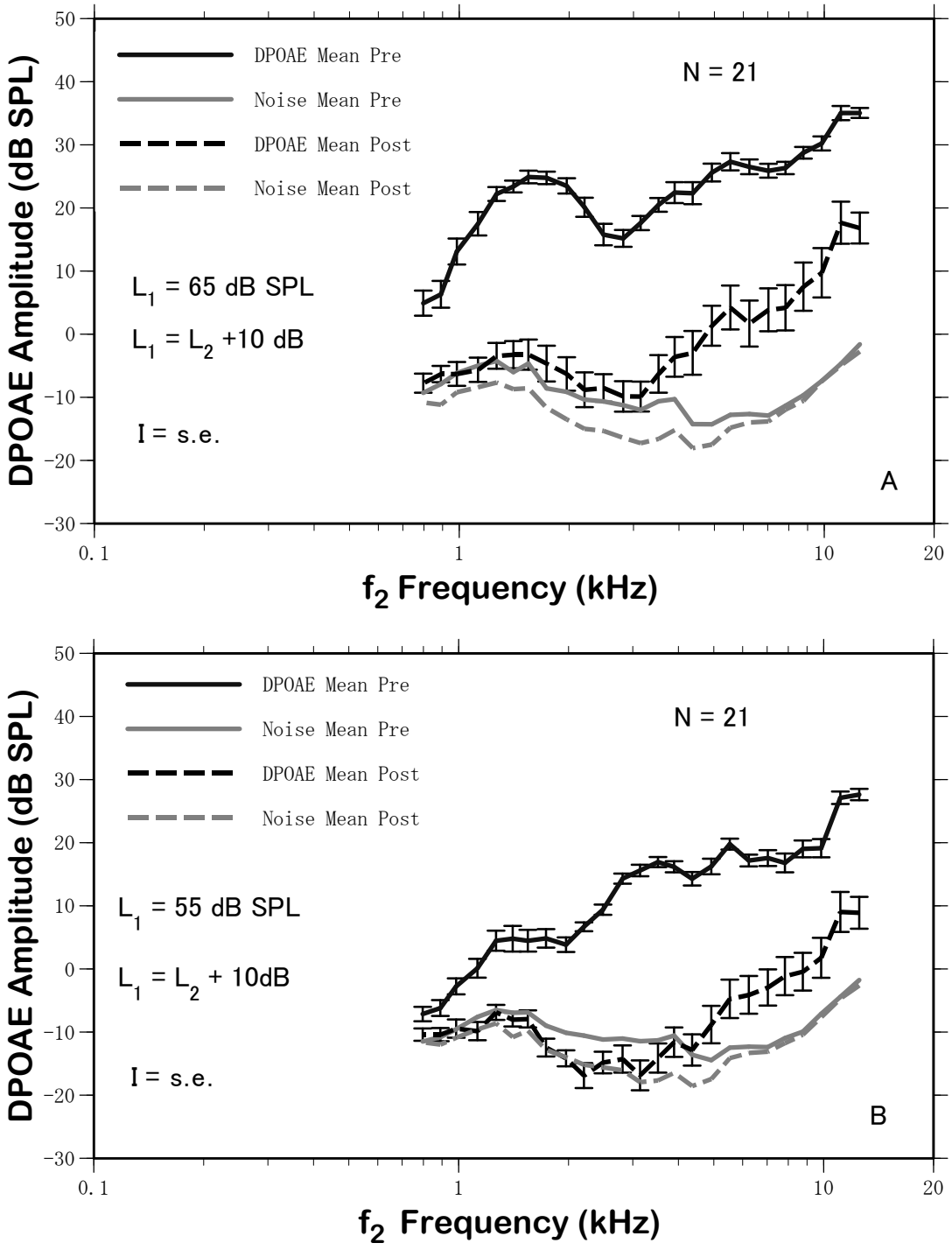


Figure 112. Phase III: Src Inh treated group mean (N = 21) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

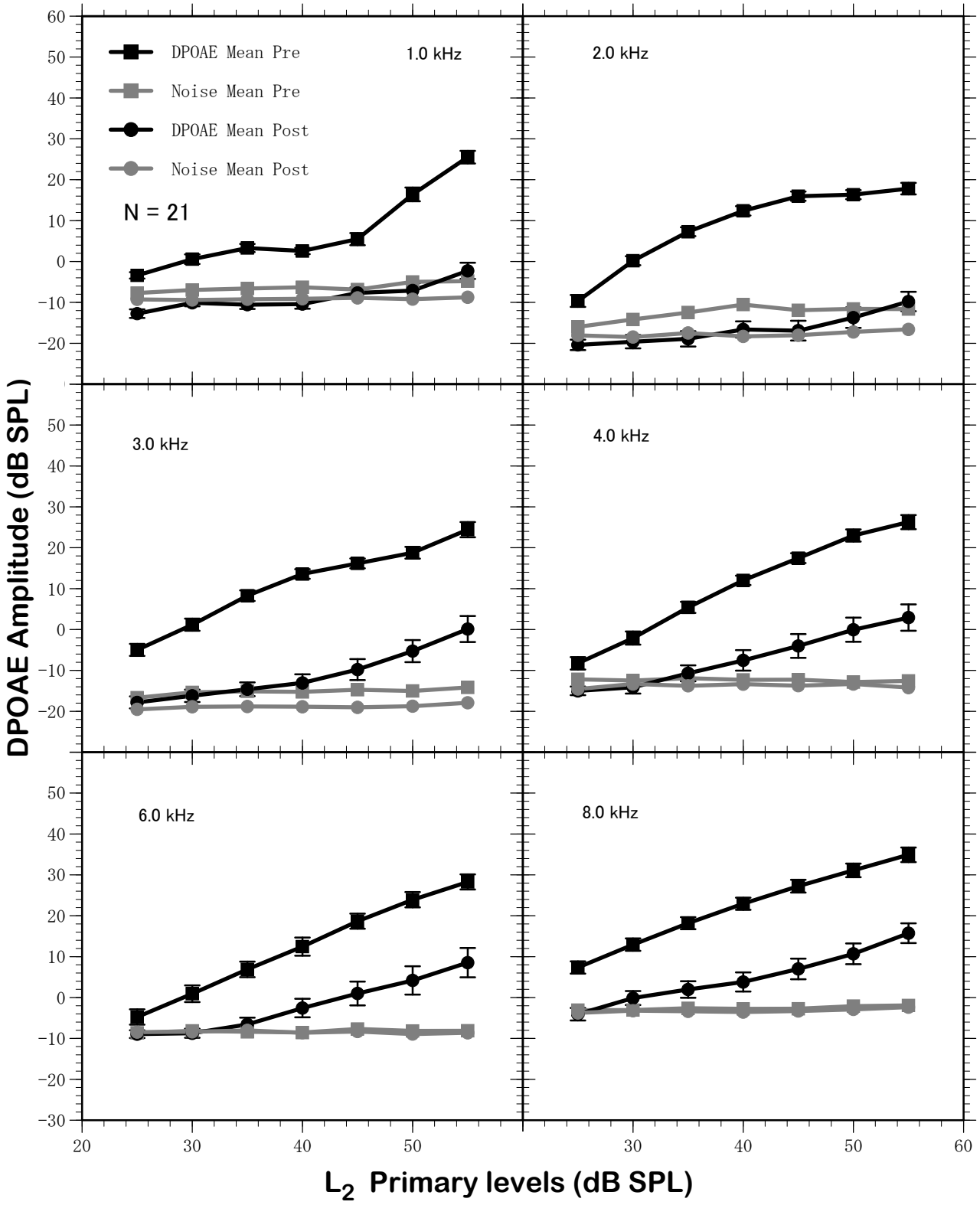


Figure 113. Phase III: Src Inh treated group mean (N=21) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

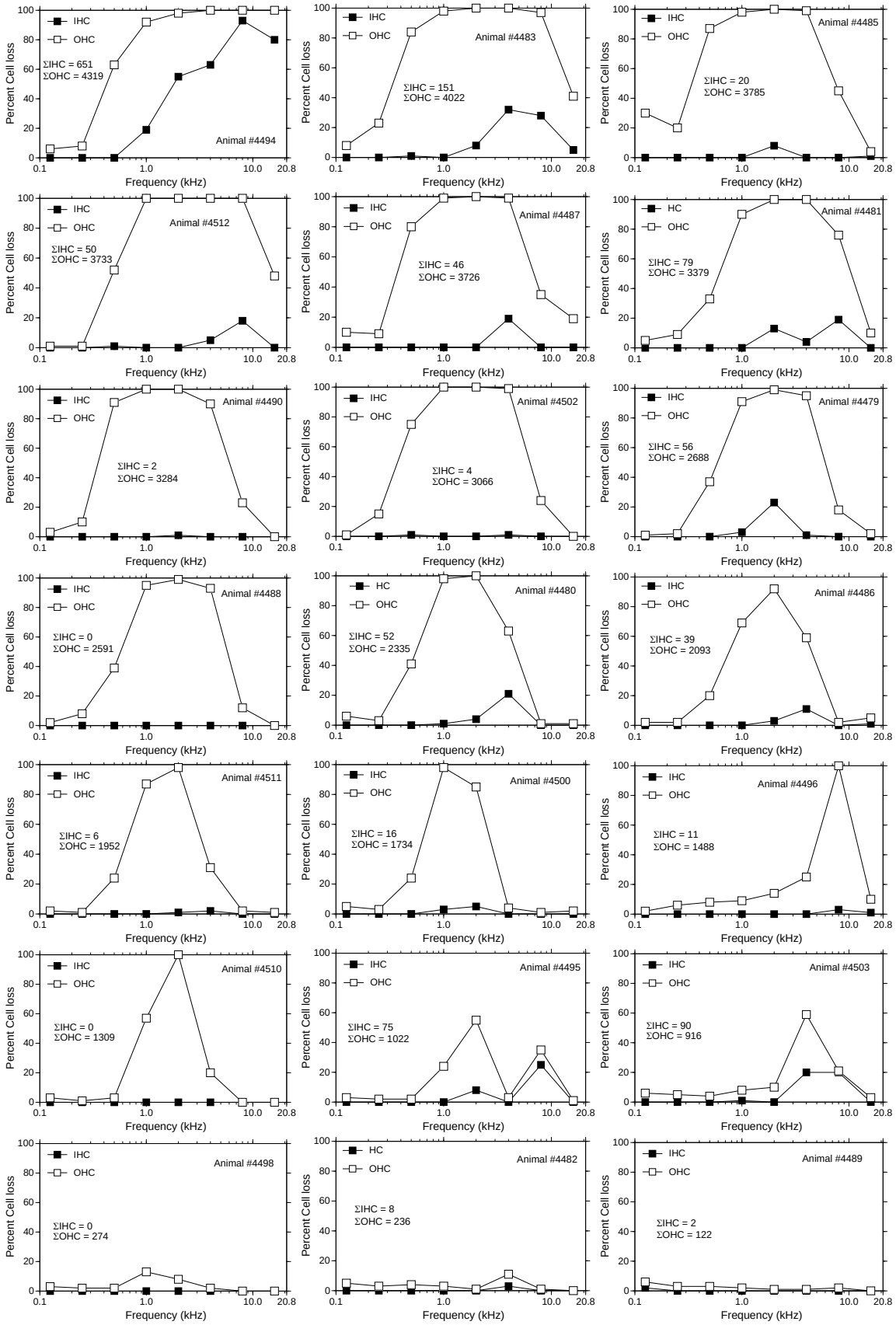


Figure 114. Phase III: Individual cochleograms for the 21 Src Inh treated animals exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Peak SPL at the subject's ear = 158 dB. Cochleograms are arranged in a decreasing order of OHC loss severity.

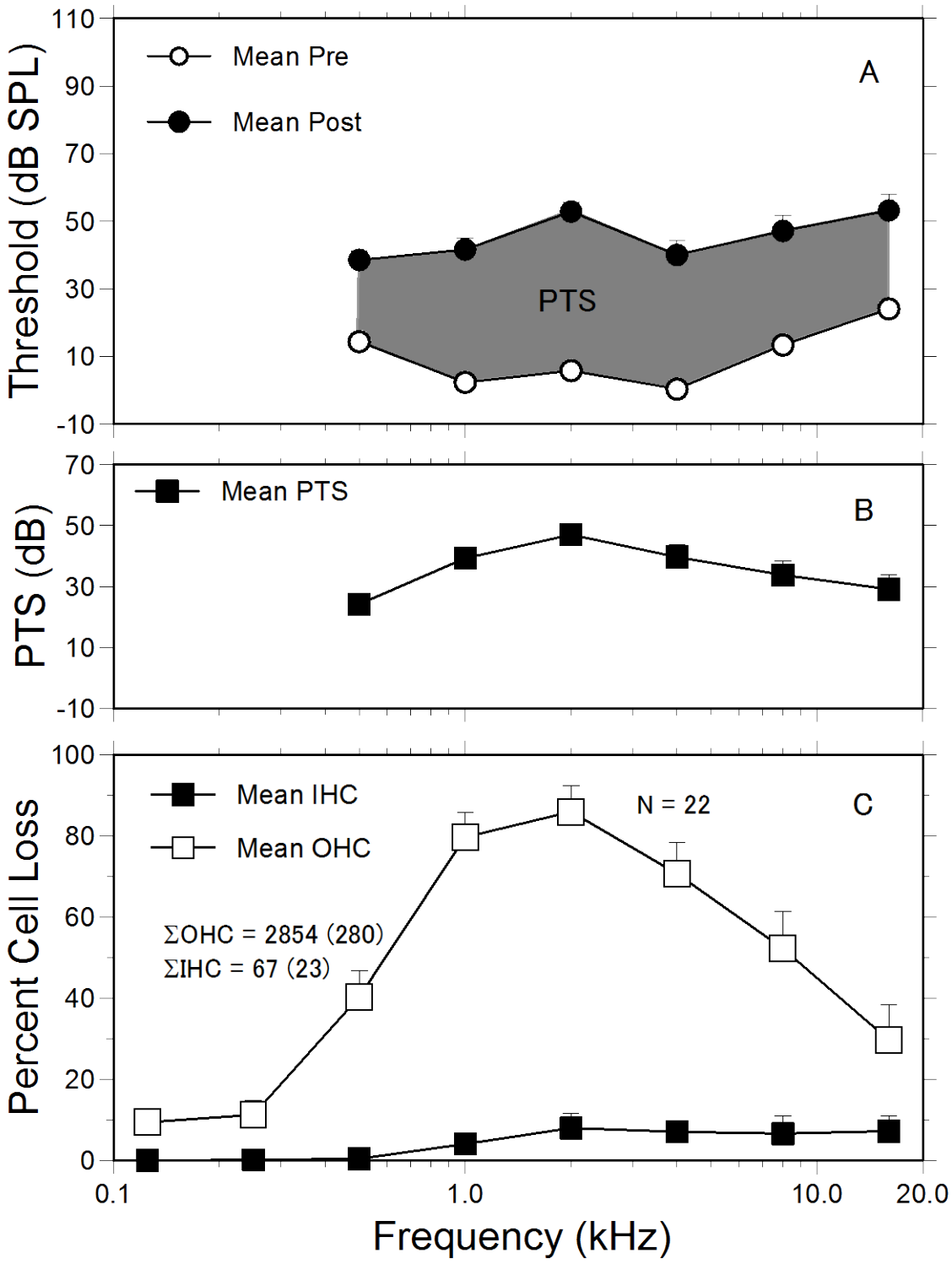


Figure 115. Phase III: Ebselen treated group (N=22) exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer hair cell (%IHC, %OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

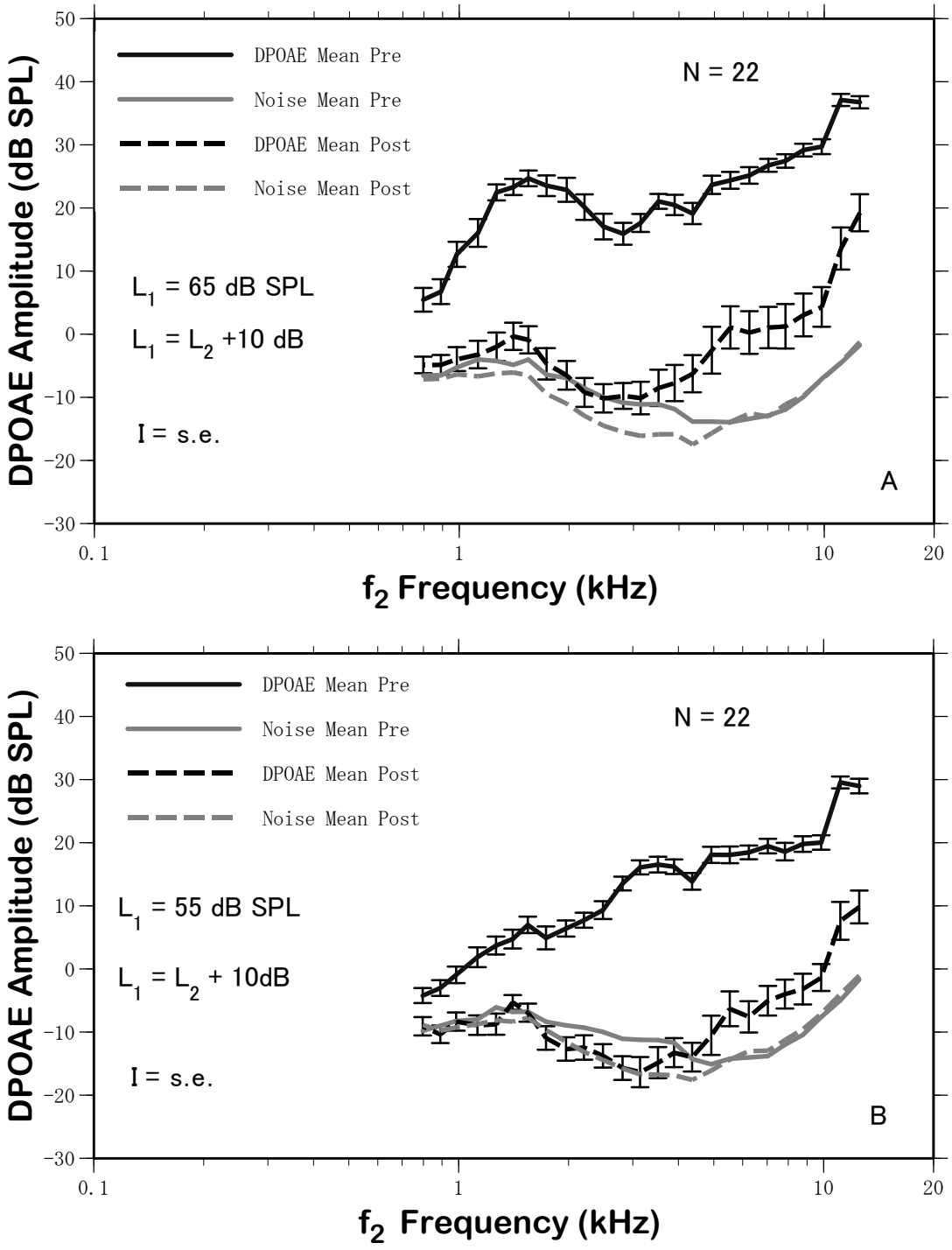


Figure 116. Phase III: Ebselen treated group mean (N=22) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

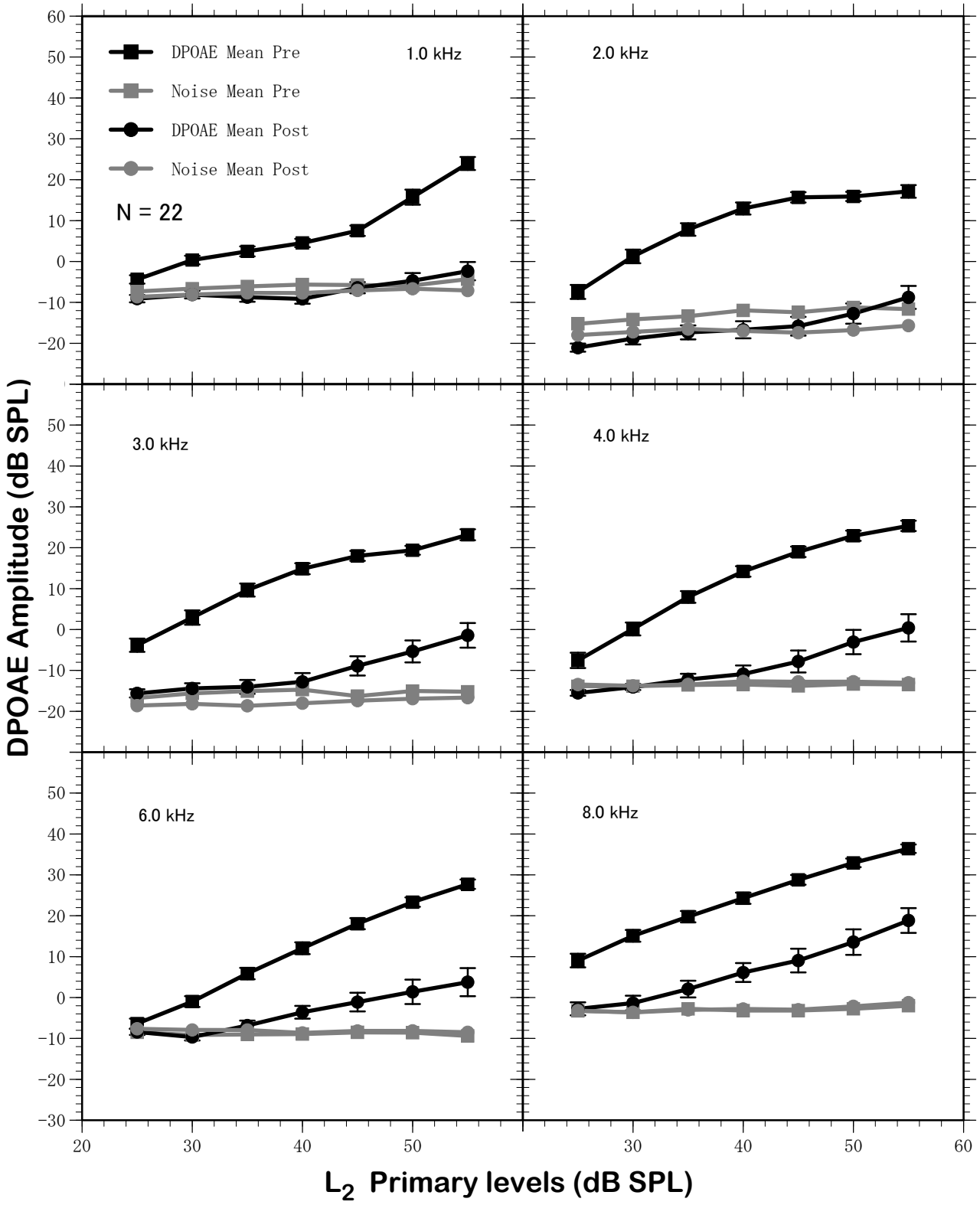


Figure 117. Phase III: Ebselen treated group mean (N=22) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (I = s.e.)

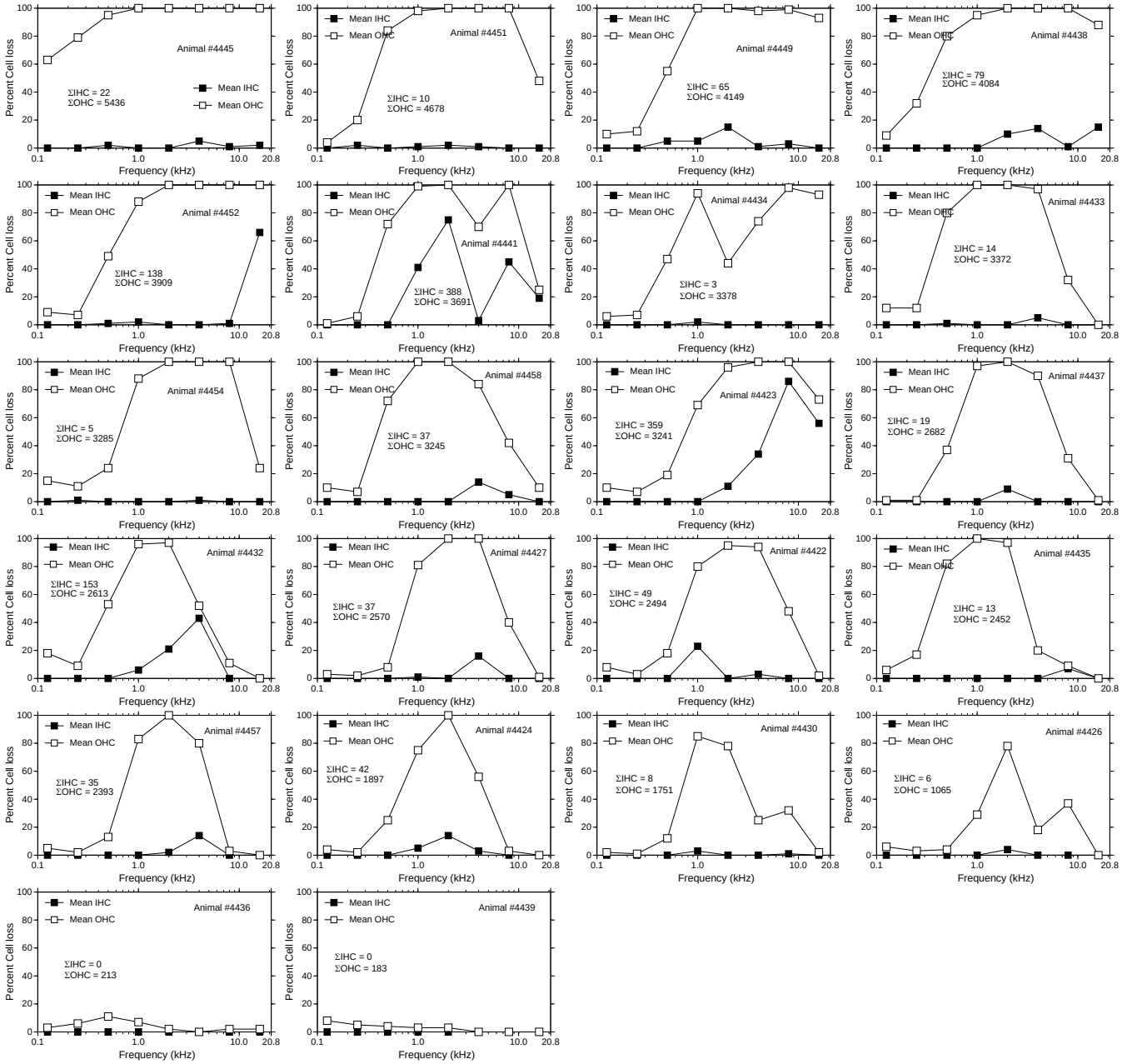


Figure 118. Phase III: Individual cochleograms for the 22 Ebselen treated animals exposed to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). Peak SPL at the subject's ear = 158 dB. Cochleograms are arranged in a decreasing order of OHC loss severity.

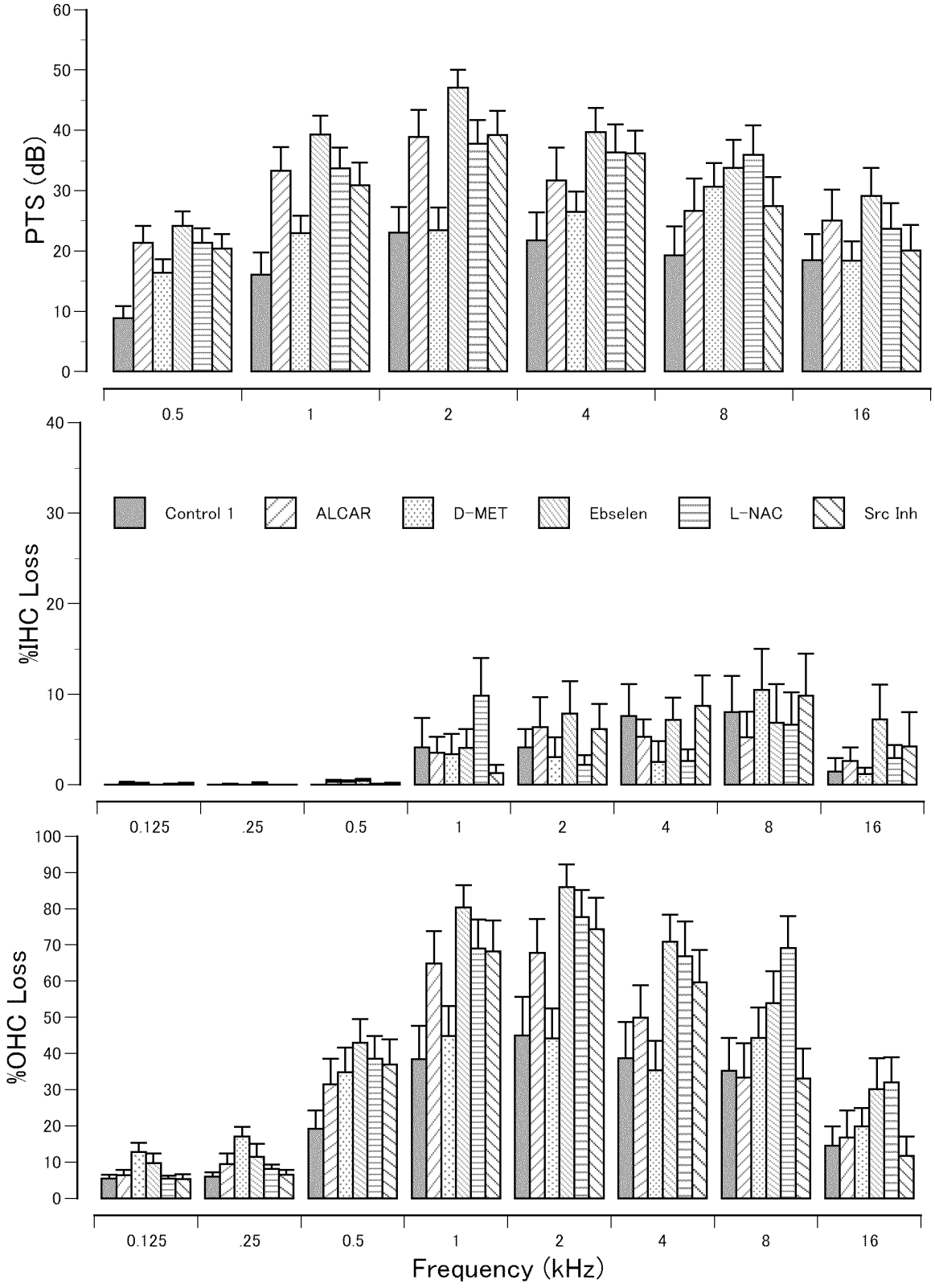


Figure 119. The PTS, %IHC and %OHC loss for the noise only control group #1 (N=20) and the five drug treated groups following exposure to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). T = standard error of the mean.

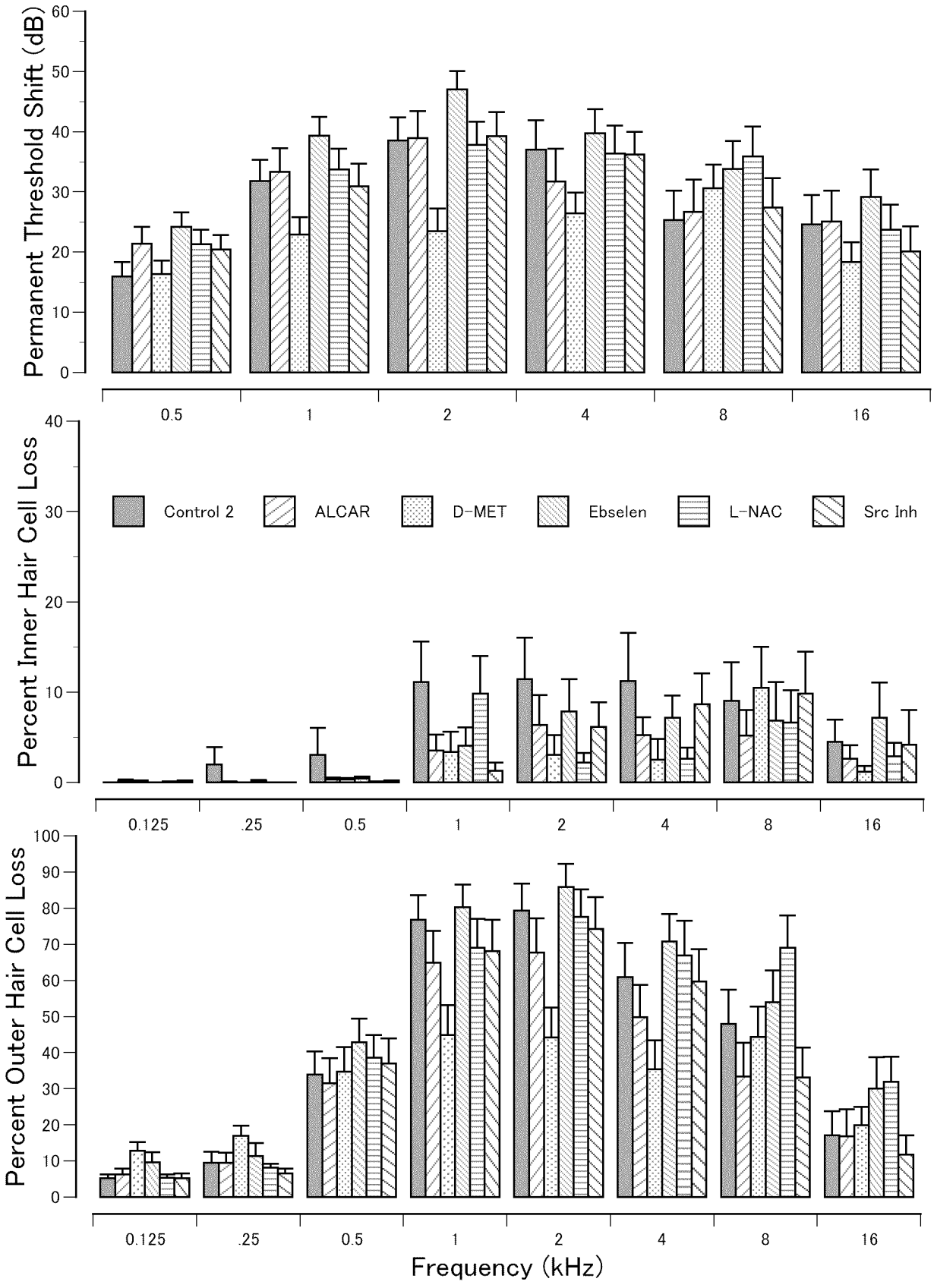


Figure 120. The mean PTS, %IHC and %OHC loss for the noise only control group #2 (N=20) and the five drug treated groups following exposure to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). T = standard error of the mean.

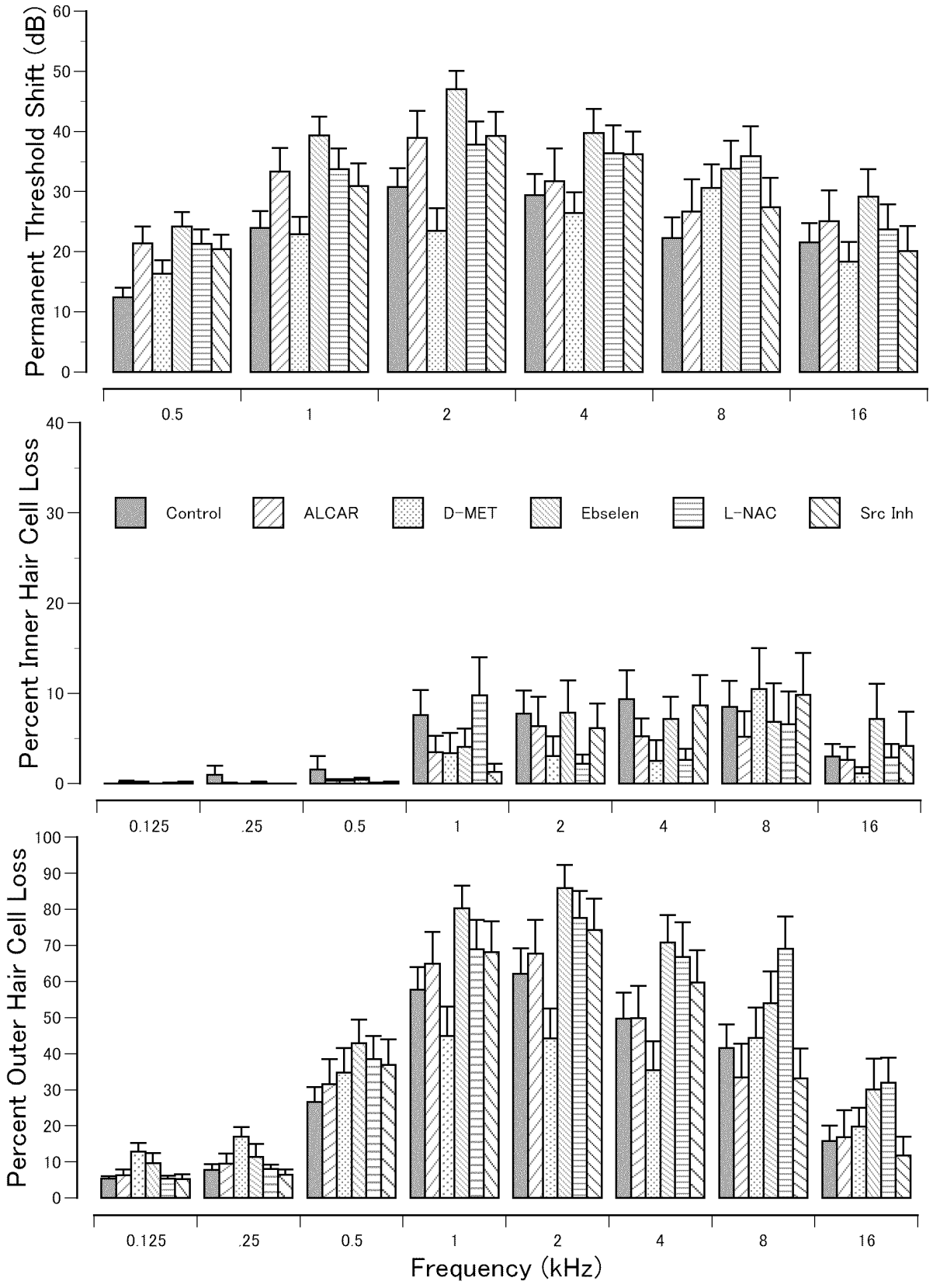


Figure 121. The PTS, %IHC and %OHC loss for the combined noise only control group (N=40) and the five drug treated groups following exposure to 10, 165 dB peak SPL free field impulses (shock tube charge pressure = 11 psi). (T = standard error of the mean)

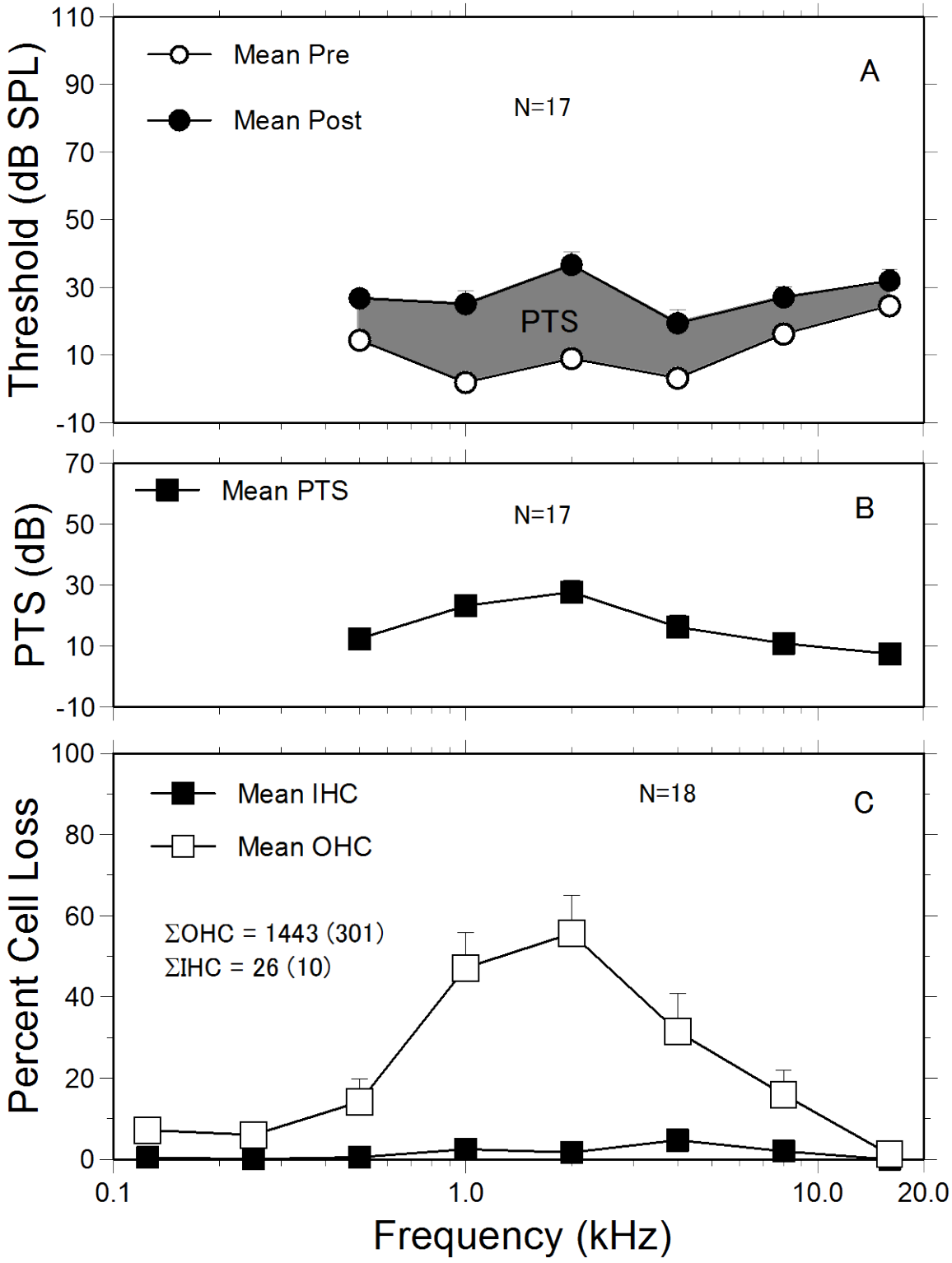


Figure 122. Phase III: Noise only control group (N=18) exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

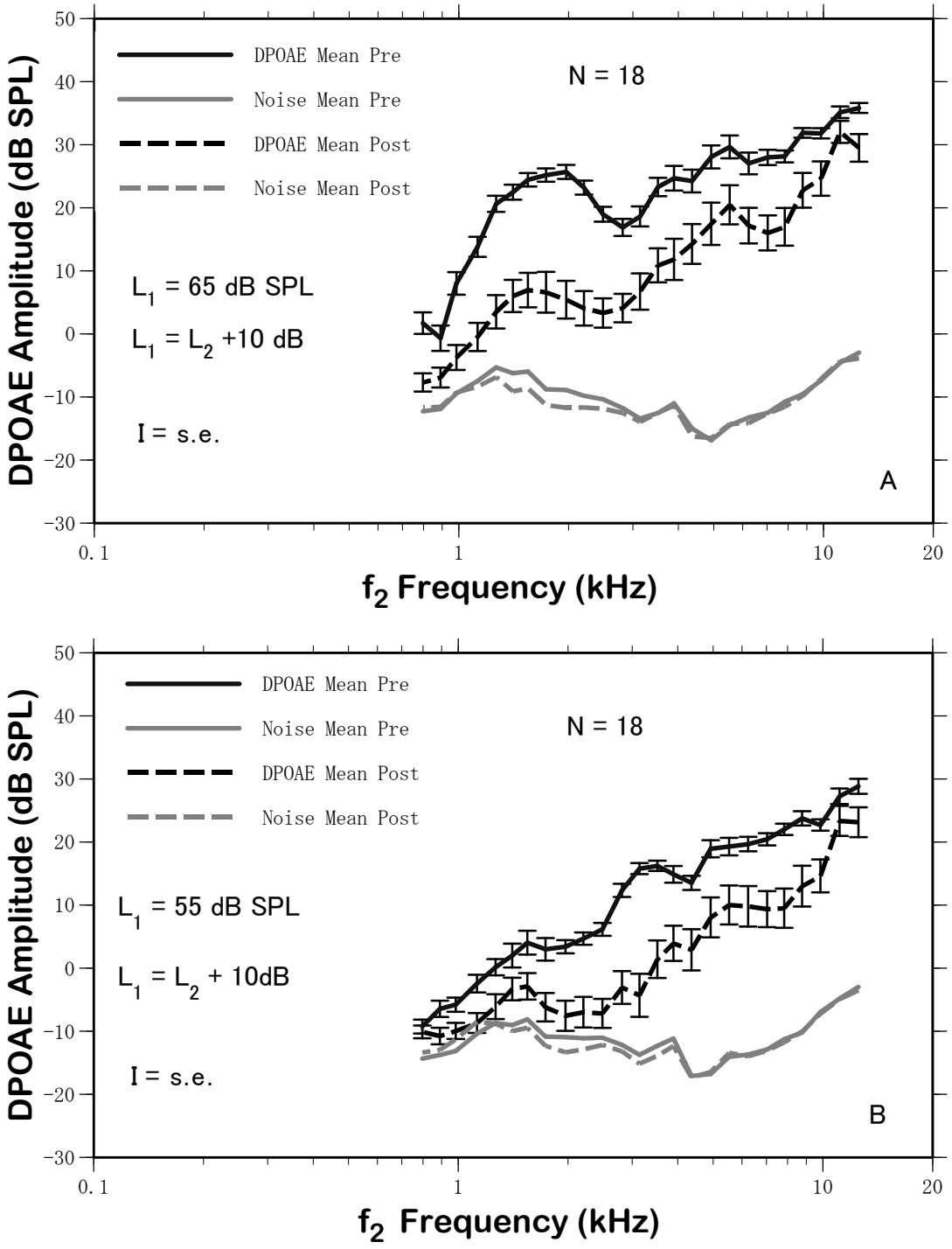


Figure 123. Phase III: Group mean (N=18) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

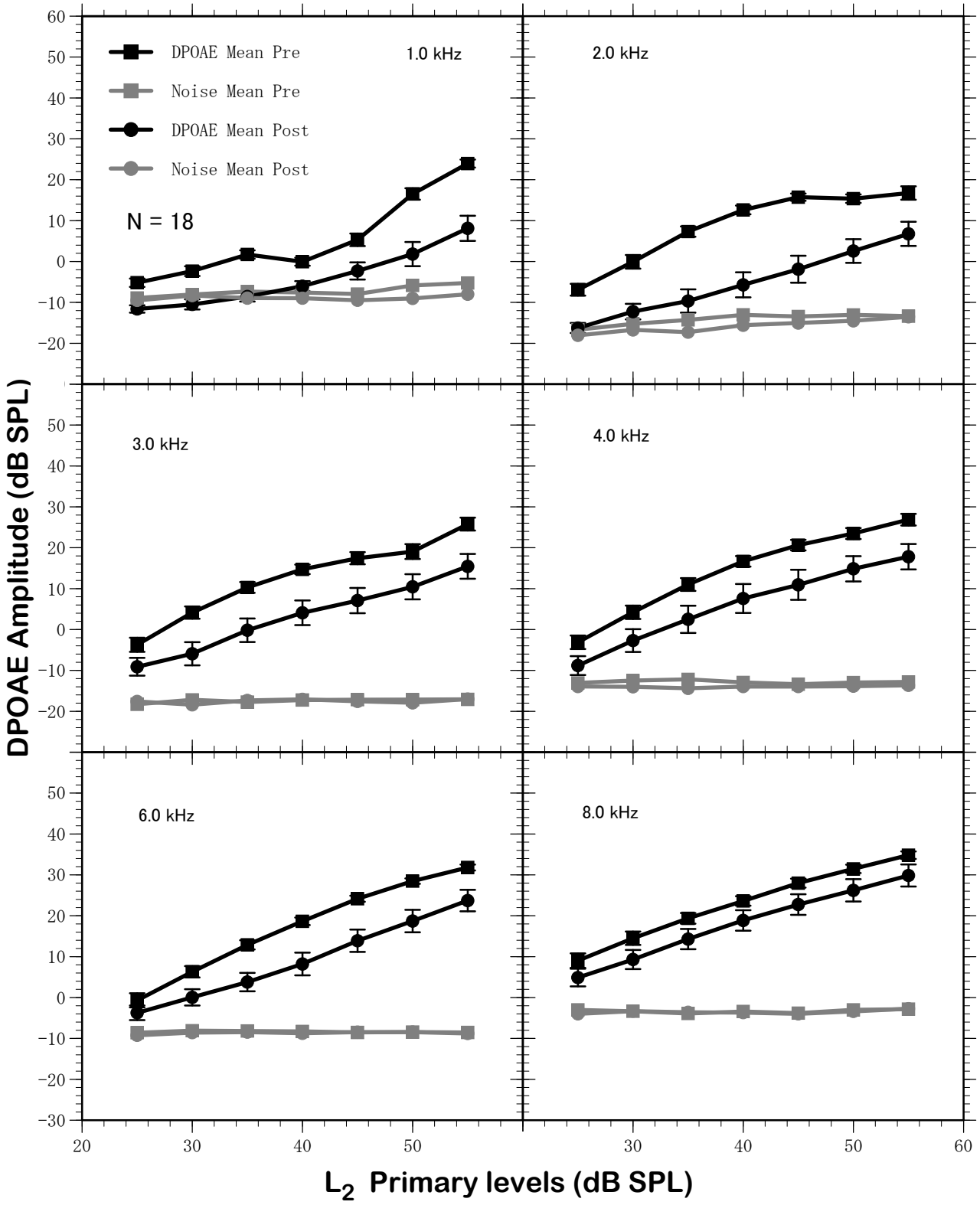


Figure 124. Phase III: Group mean (N=18) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

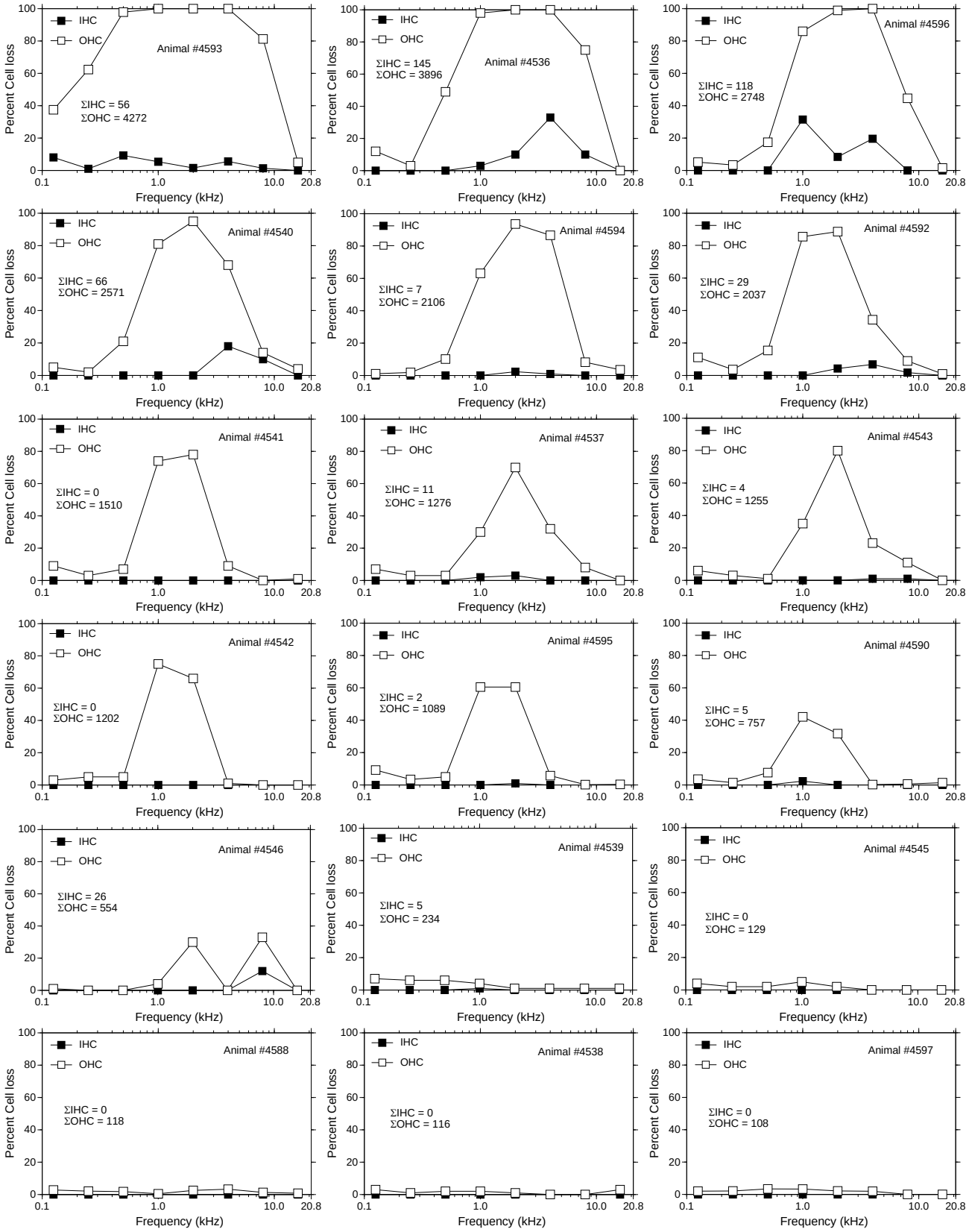


Figure 125. Phase III: Individual cochleograms for the 18 noise only control animals exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Peak SPL at the subject's ear = 156 dB. Cochleograms are arranged in a decreasing order of OHC loss severity.

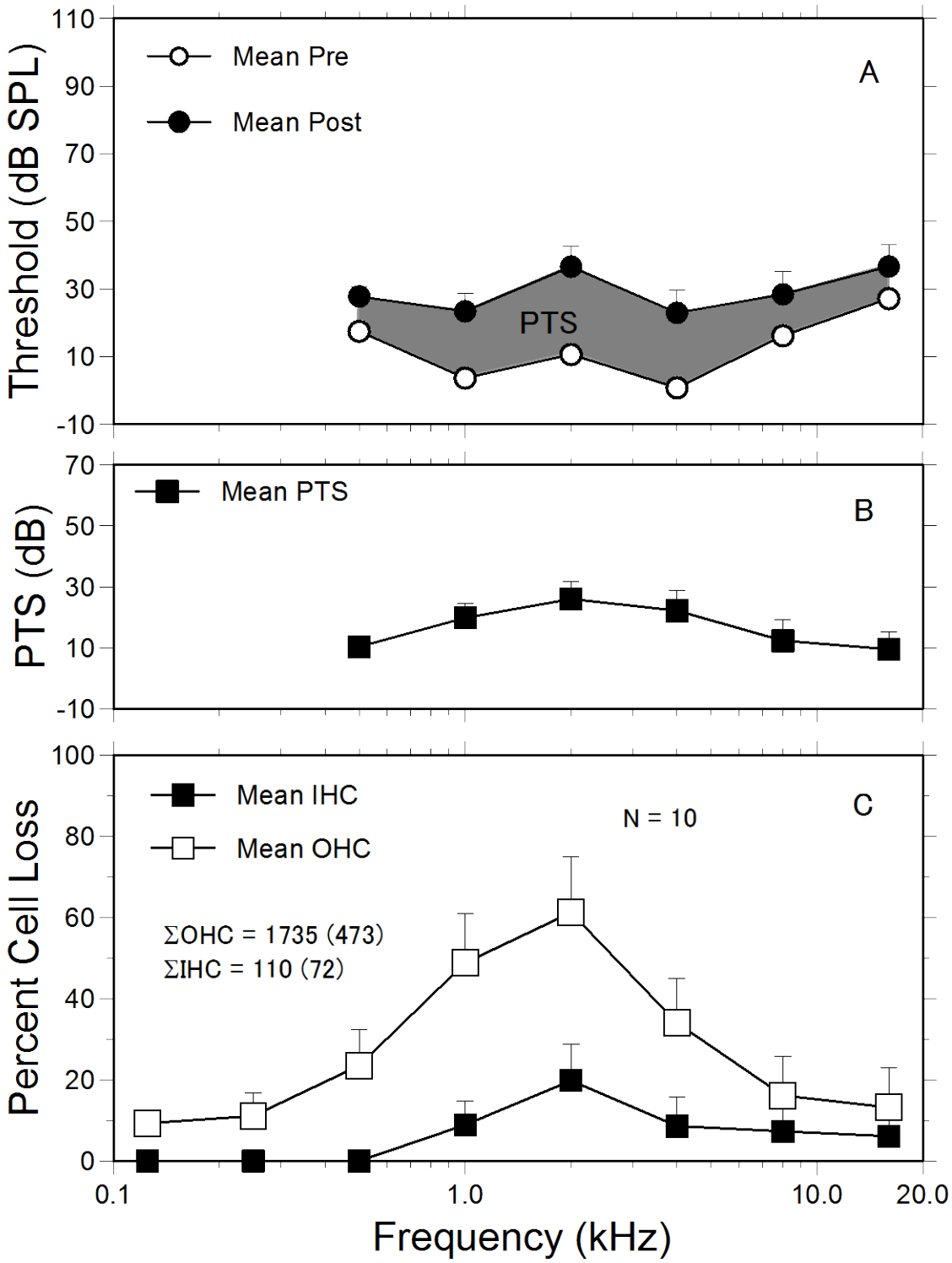


Figure 126. Phase III: L-NAC treated group (N=10) exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

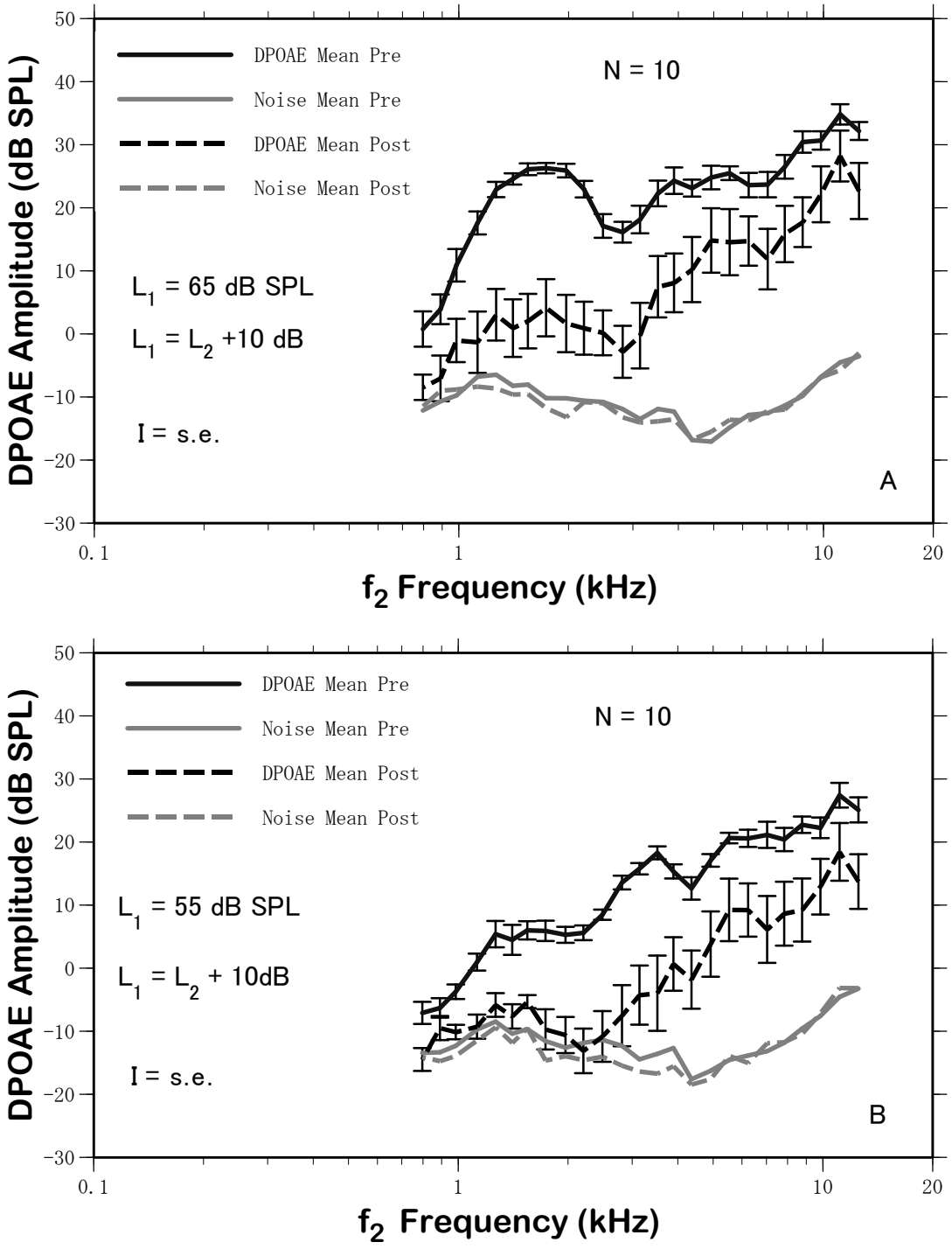


Figure 127. Passe III: L-NAC treated group mean (N=10) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

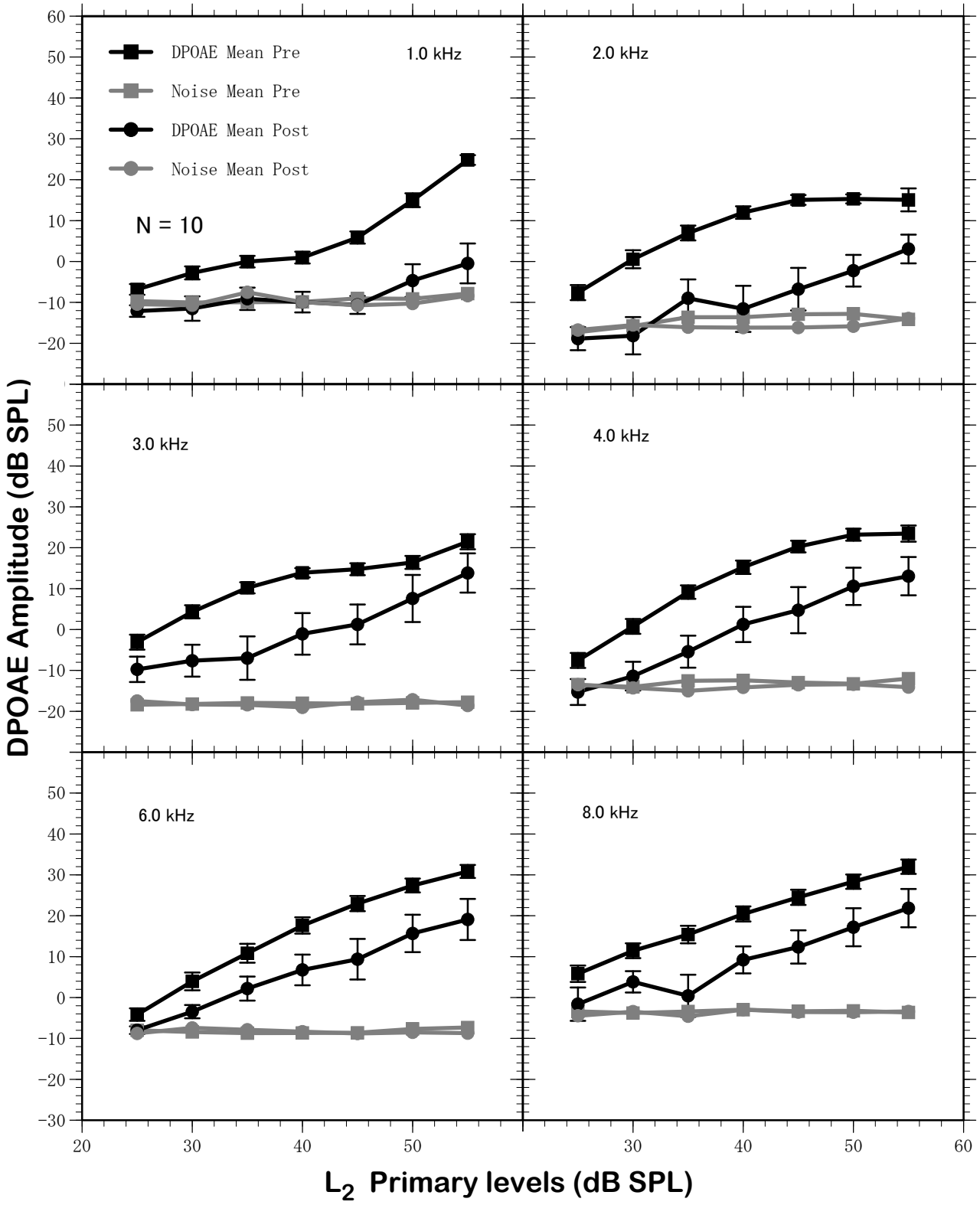


Figure 128. Phase III: L-NAC treaded group mean (N=10) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

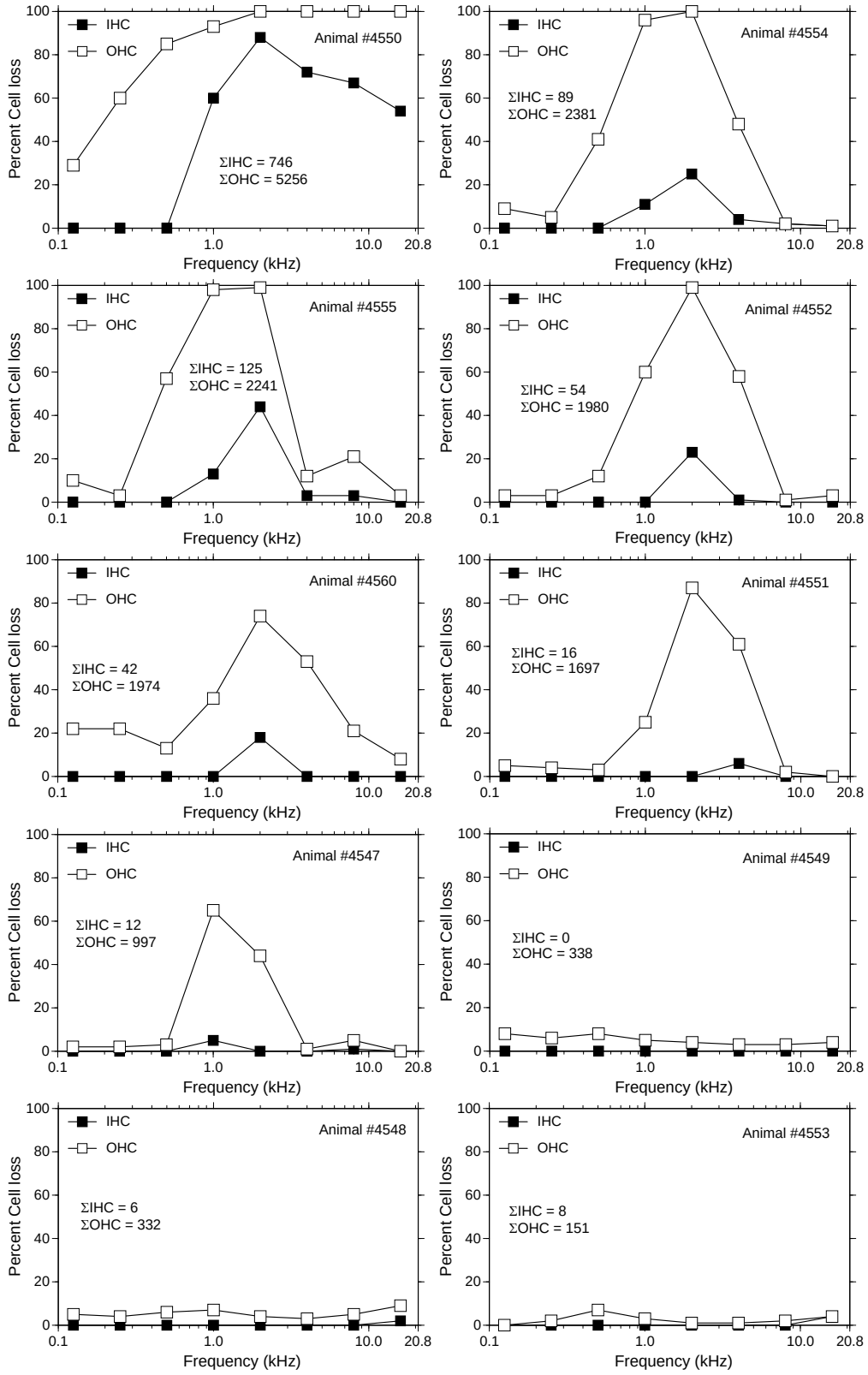


Figure 129. Phase III: Individual cochleograms for L-NAC treated animals exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Peak SPL at the subject's ear = 156 dB. Cochleograms are arranged in a decreasing order of OHC loss severity.

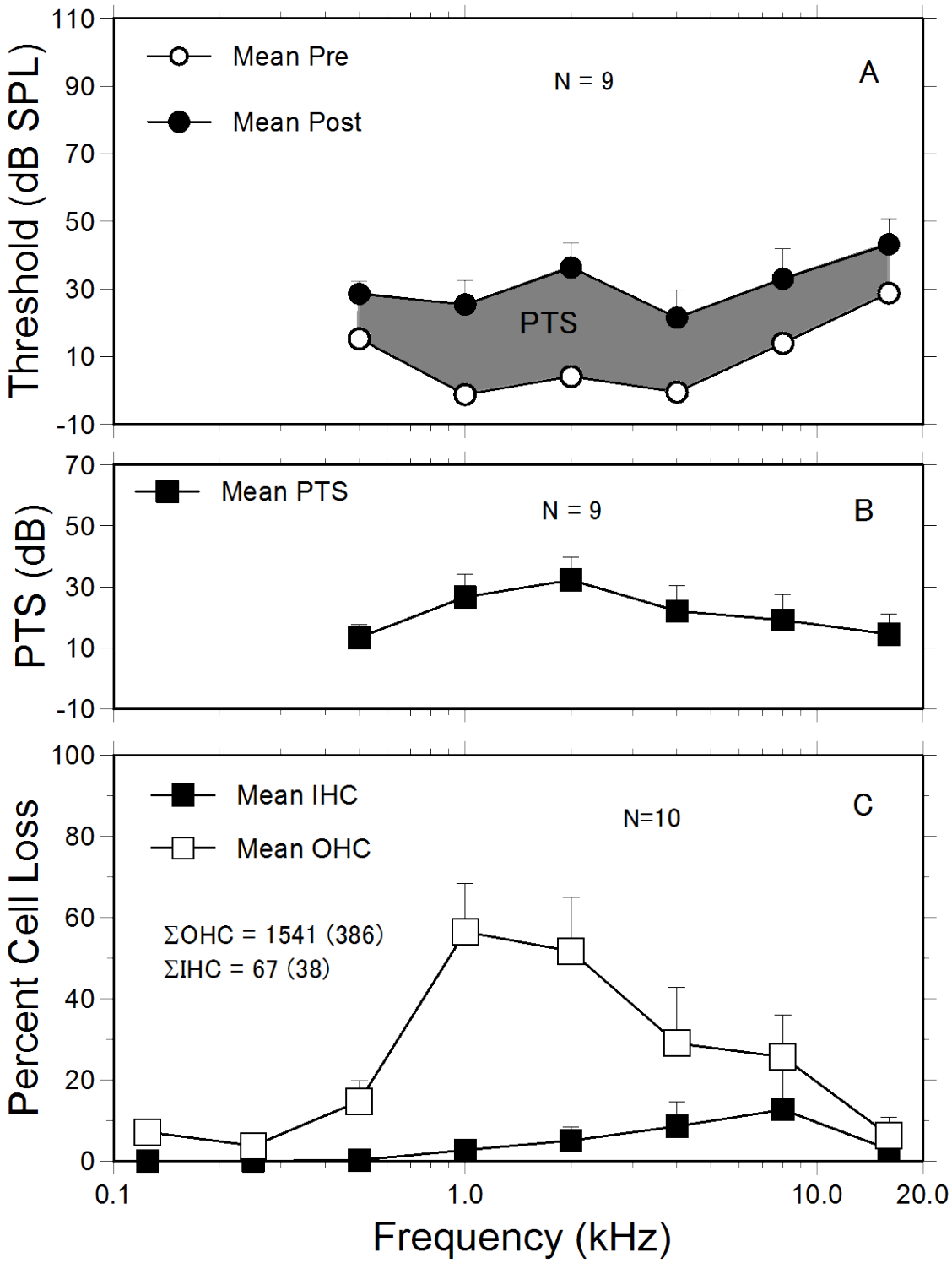


Figure 130. Phase III: ALCAR treated group (N=10) exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS). (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

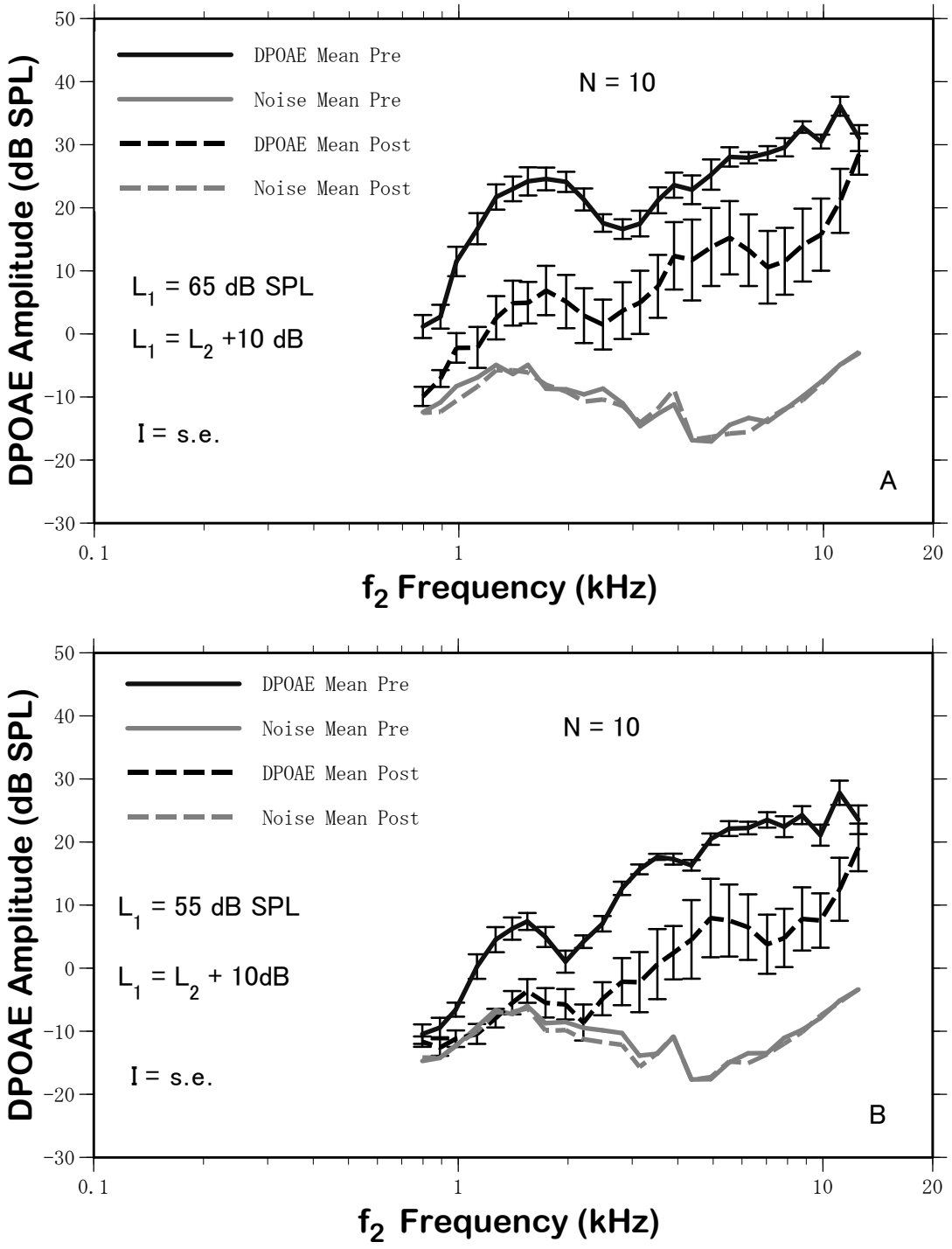


Figure 131. Phase III: ALCAR treated group mean (N=10) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

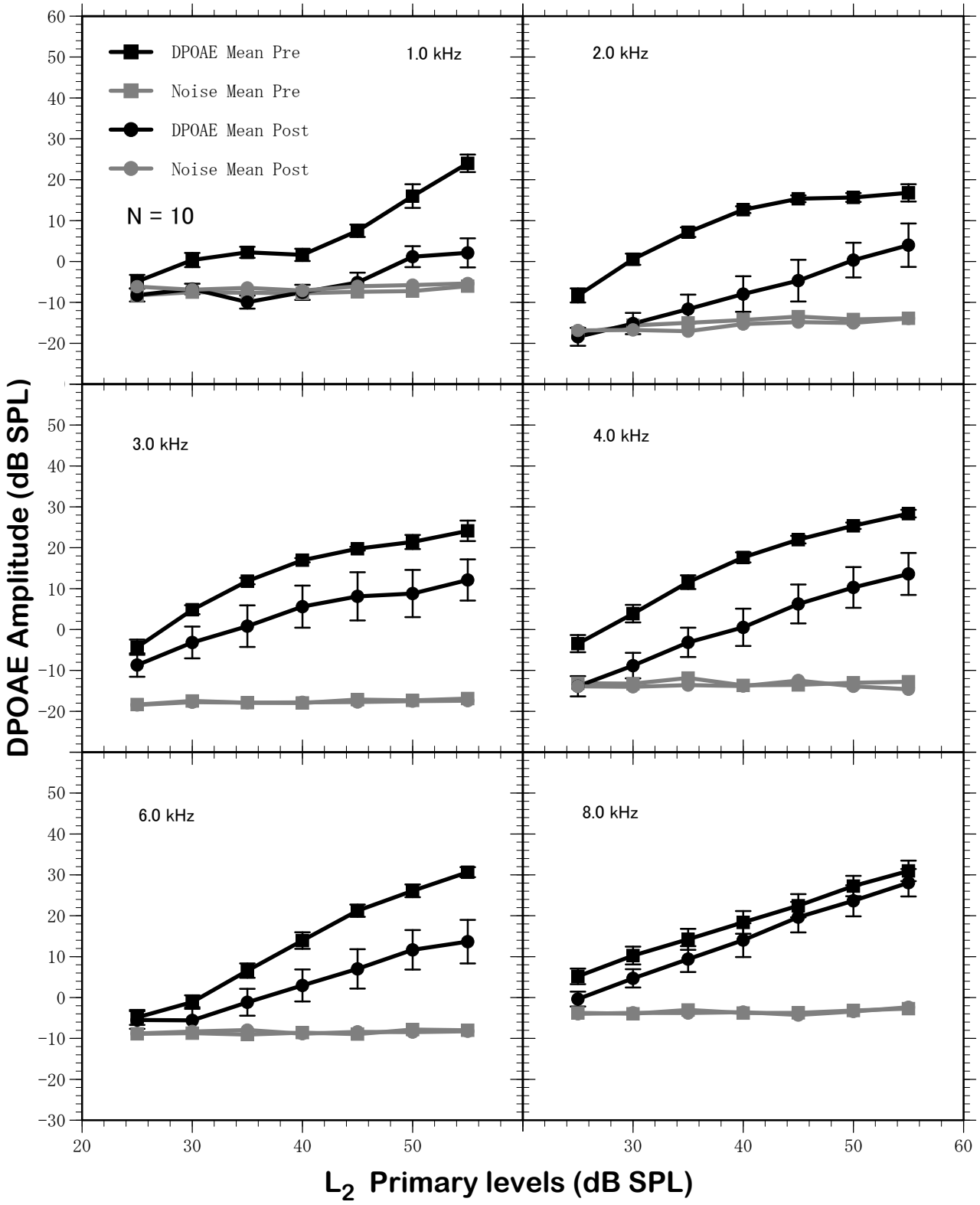


Figure 132. Phase III: ALCAR treated group mean (N=10) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

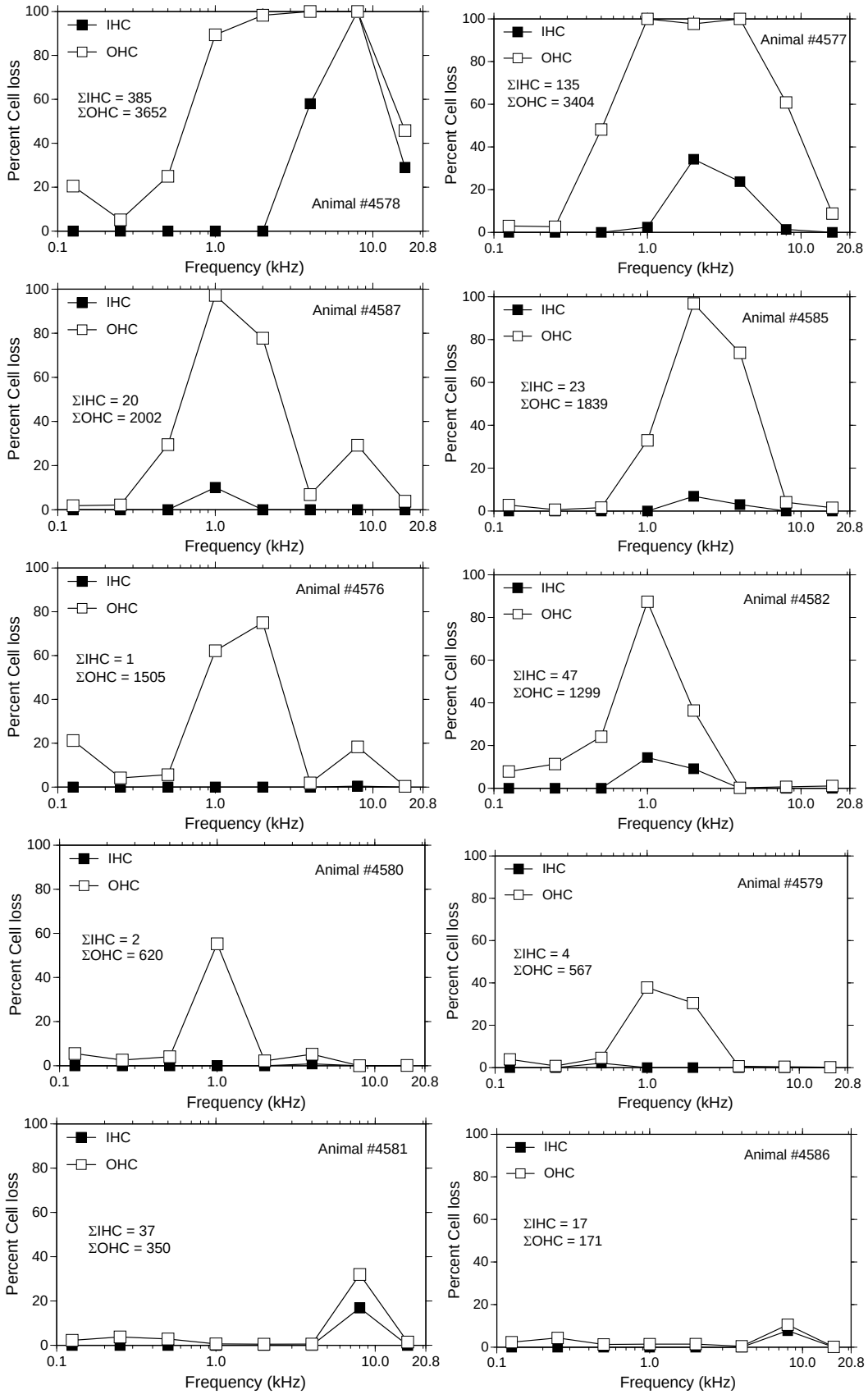


Figure 133. Phase III: Individual cochleograms for ALCAR treated animals exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Peak SPL at the subject's ear = 156 dB. Cochleograms are arranged in a decreasing order of OHC loss severity.

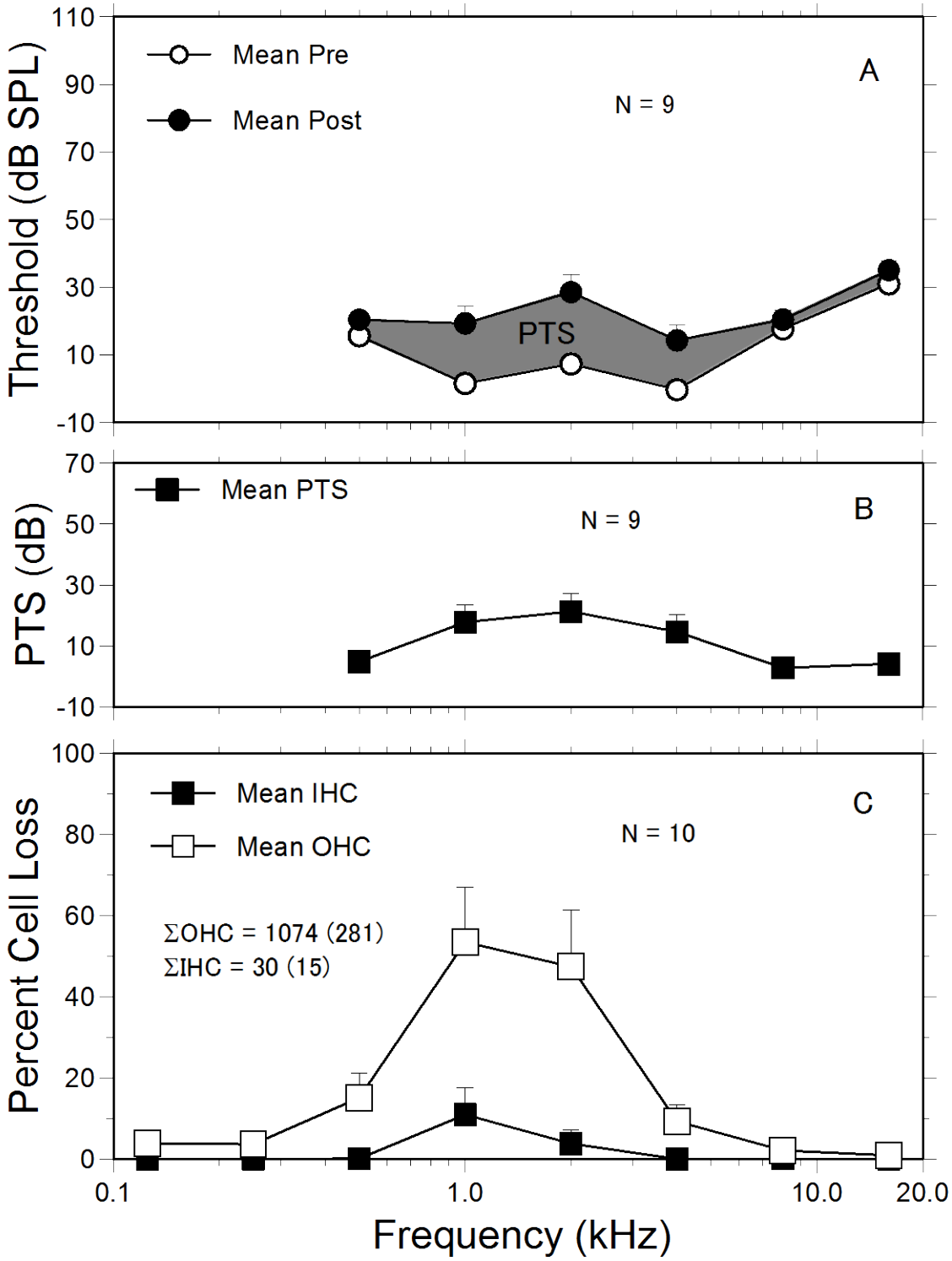


Figure 134. Phase III: D-MET treated group (N=10) exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Group mean (A) pre and post exposure audiograms. Shaded area indicates permanent threshold shift (PTS) (B) PTS and (C) percent inner and outer haircell (IHC, OHC) loss. ΣOHC and ΣIHC = total number of missing sensory cells, with standard error (s.e.) of the mean in parentheses. (T = s.e.)

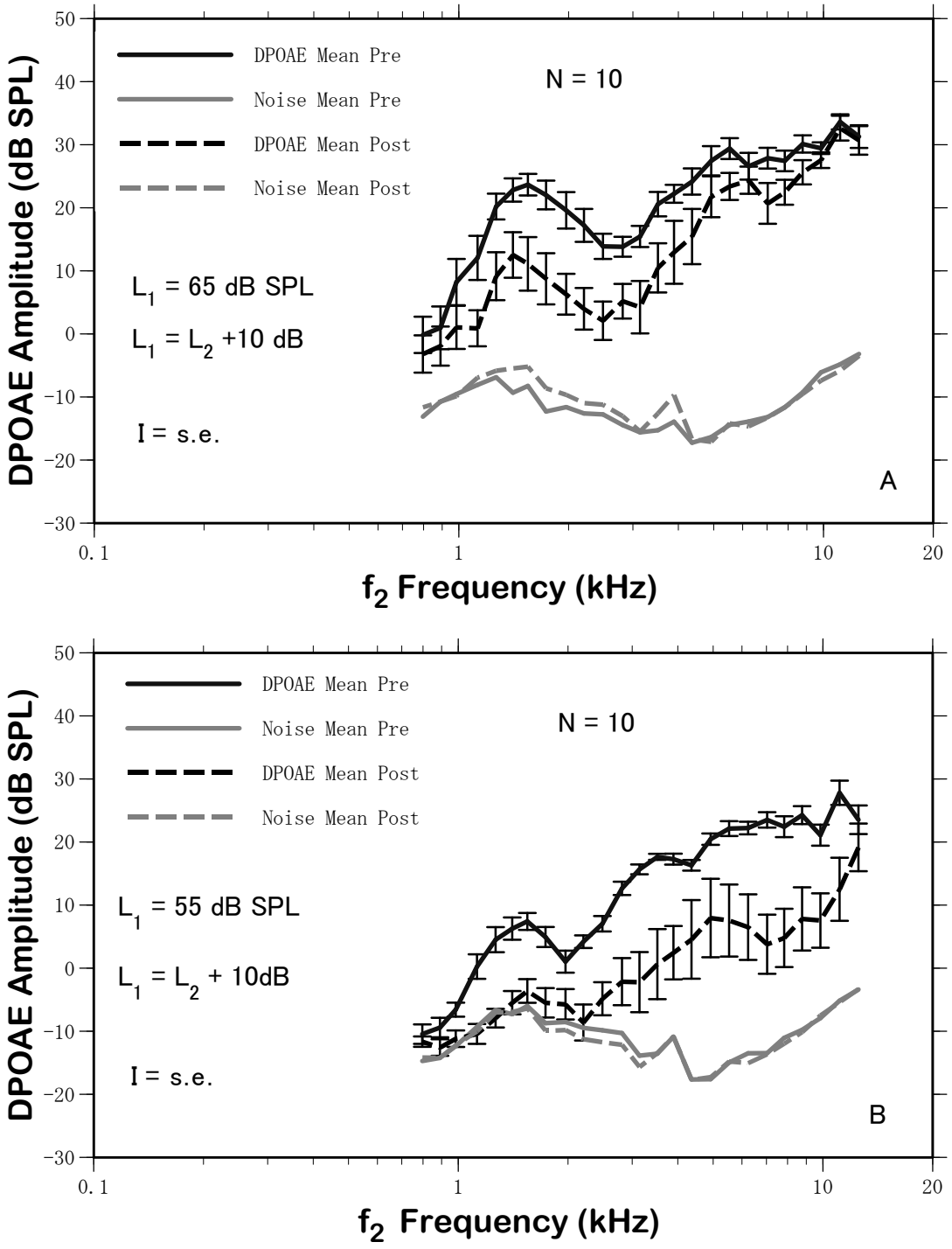


Figure 135. Phase III: D-MET treated group mean (N=10) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) as a function of the f_2 primary frequency. The DPOAE is measured using upper and lower primary frequencies f_2 and f_1 having levels L_2 and L_1 respectively. (A) L_1 = 65 dB SPL and (B) L_1 = 55 dB SPL where $L_1 = L_2$+10 dB and f_2/f_1 = 1.22. Exposure: 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

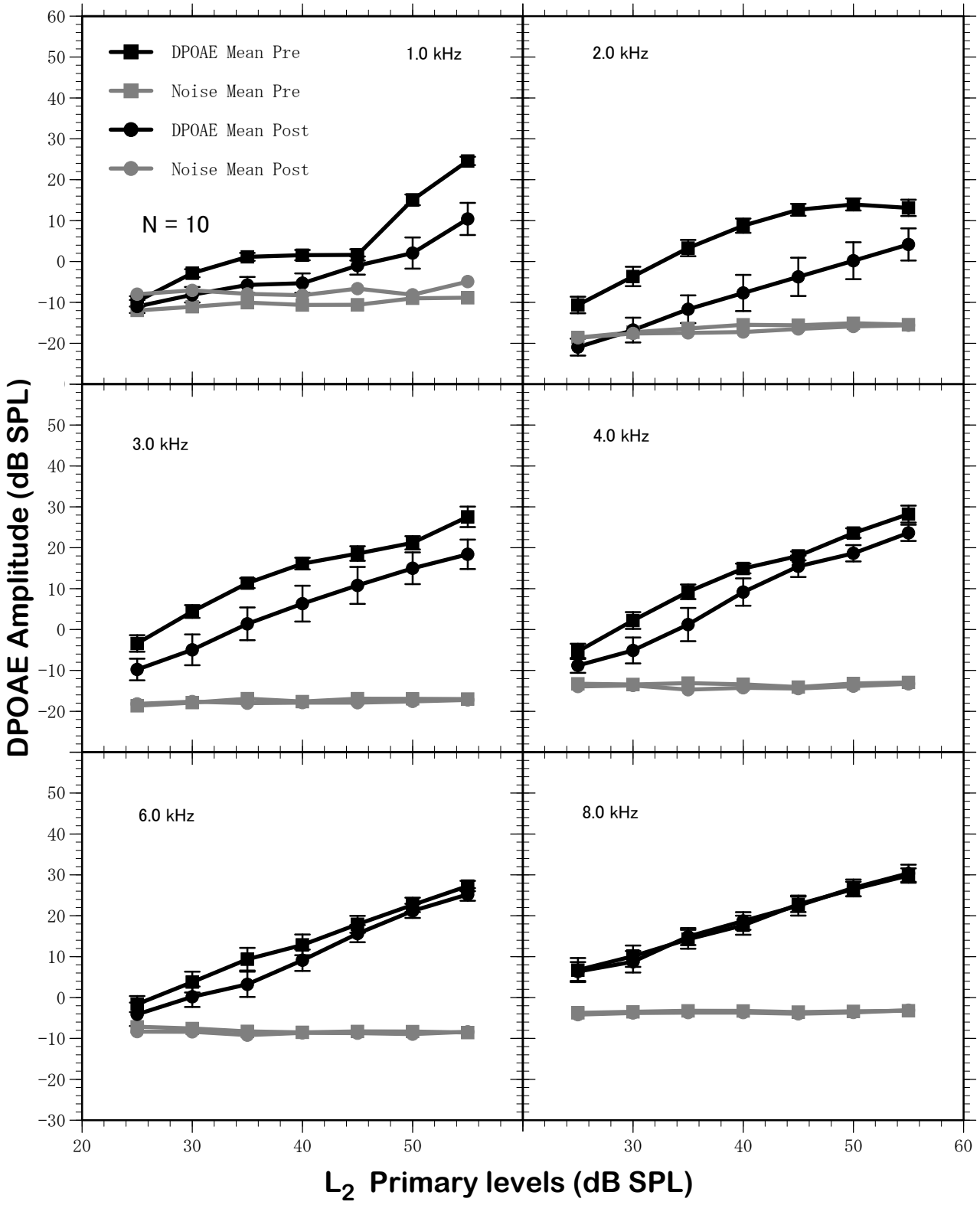


Figure 136. Phase III: D-MET treated group mean (N=10) pre and post exposure cubic ($2f_1$-f_2) distortion product otoacoustic emission (DPOAE) input/output functions measured at the approximate frequencies indicated as a function of the level L_2 of the upper primary frequency f_2. Exposure:10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). (I = s.e.)

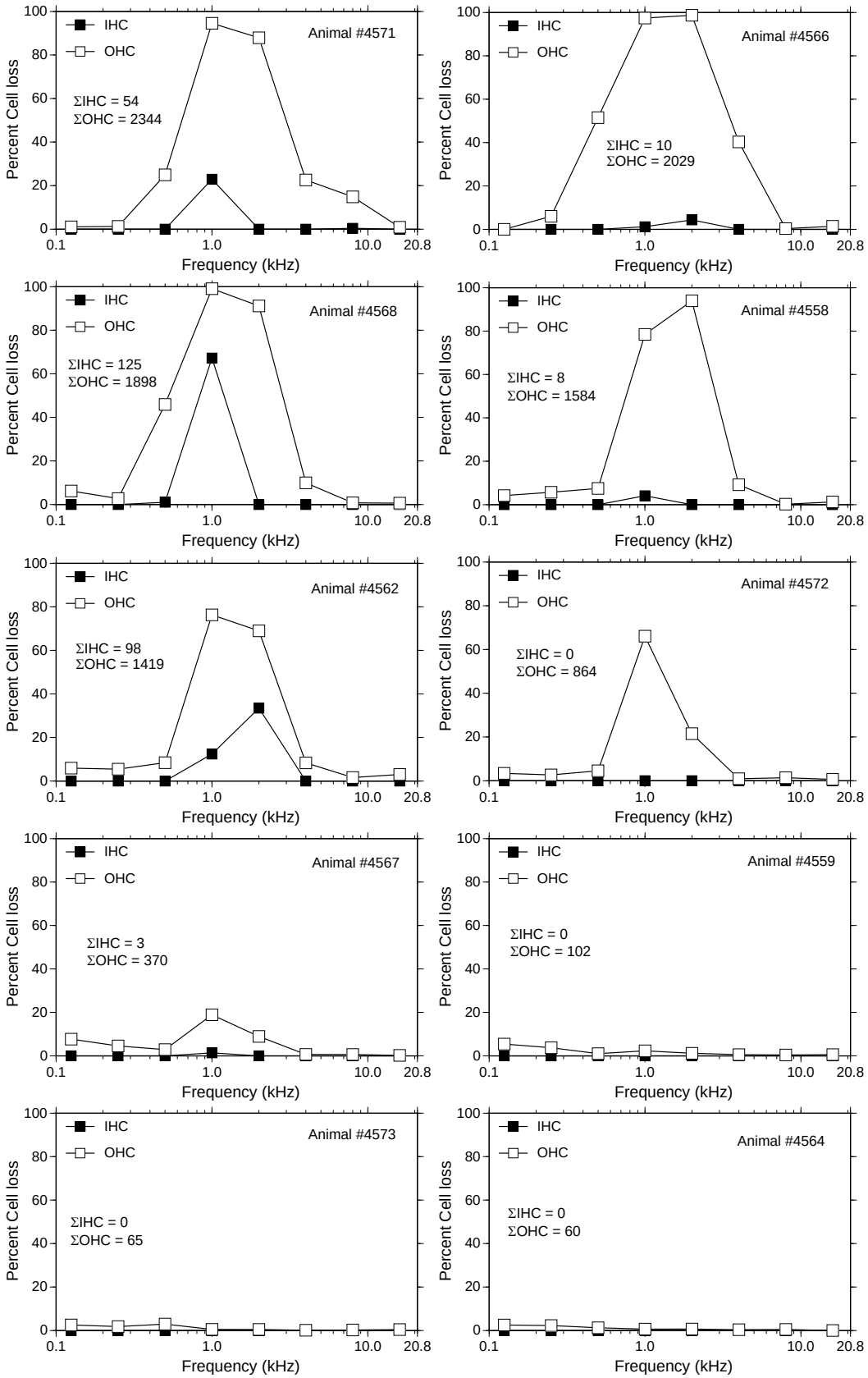


Figure 137. Phase III: Individual cochleograms for D-MET treated animals exposed to 10, 162 dB peak SPL free field impulses (shock tube charge pressure = 7 psi). Peak SPL at the subject's ear = 156 dB. Cochleograms are arranged in a decreasing order of OHC loss severity.

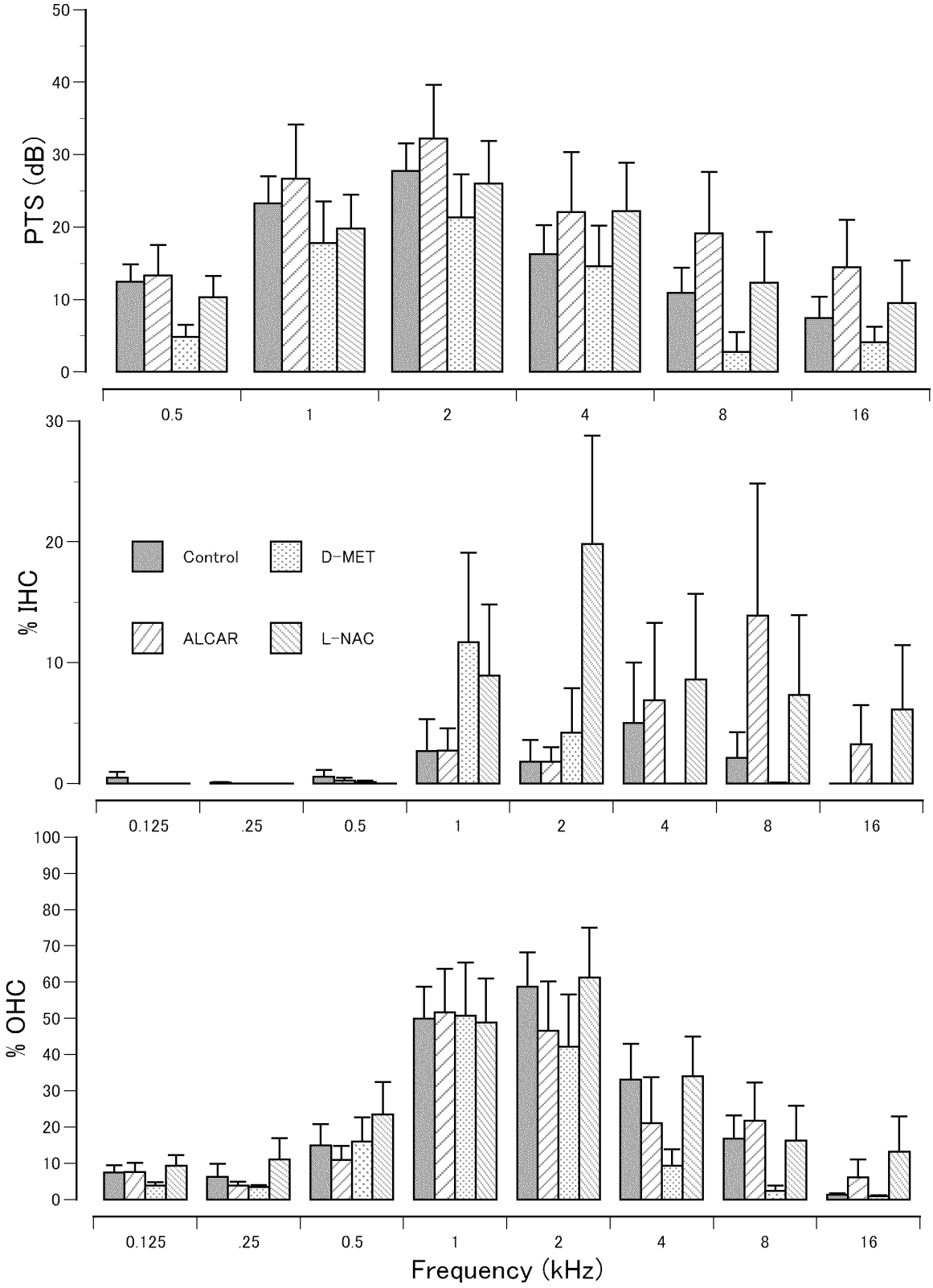


Figure 138. The mean PTS, %IHC and %OHC loss for the noise only control group and the three drug treated groups following exposure to 10, 162 dB peak SPL free field impulse (shock tube charge pressure = 7 psi). T = standard error of the mean.

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VII. Appendix:

Abbreviated Vitae for Roger P. Hamernik, Ph.D.

(1) Education:

Syracuse University	B.S	June 1963
Syracuse University	M.S. (Mechanical Eng.)	January 1967
Syracuse University	Ph.D. (Mechanical Eng.)	June 1970

(2) Recent Professional Appointments:

Professor, State University of New York at Plattsburgh, Departments of Speech and Hearing and Physics. (January 1986-present)
Professor, University of Texas at Dallas, Callier Center for Communication Disorders (June 1980-Dec. 1985)
Associate Professor, Dept. Otolaryngology and Communication Sciences, SUNY, Upstate Med. Center, Syracuse, NY (Sept. 1970-June 1980)

(3) Books:

Henderson, D., Hamernik, R.P., Dosanjh, D.S. and Mills, J. (eds), (1976). Effects of Noise on Hearing. Raven Press, NY.
Hamernik, R.P., Henderson, D. and Salvi, R. (eds), (1982). New Perspectives on Noise Induced Hearing Loss. Raven Press, NY.
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